Clinical Study Protocol RELAX (TUD-RELAX1-070)

Phase-I/II trial for relapsed or refractory AML patients combining cytarabine and mitoxantrone with venetoclax (RELAX)

Version 3-0, 20. September 2019

Authors: Christoph Röllig, Johannes Schetelig, Martin Wermke, Michael Kramer, Martin Bornhäuser

Sponsor
Technische Universität Dresden, Germany

Sponsor Code TUD-RELAX1-070

EudraCT Number 2018-003025-28

Coordinating Investigator (Leiter der klinischen Prüfung, LKP)
Prof. Dr. med. Christoph Röllig

Medizinische Klinik und Poliklinik I, Medizinische Fakultät der Technischen Universität Dresden, Fetscherstr. 74, 01307 Dresden christoph.roellig@uniklinikum-dresden.de

Confidentiality

Table of Contents

List of Tables

List of Figures

List of abbreviations

AE	Adverse Event
ALAT	Alaninaminotransferase
AMG	Arzneimittelgesetz (German Medicines Act)
AML	Acute Myeloid Leukemia
ANC	Absolute Neutrophil Count
AP	Alkaline phosphatase
APL	Acute Promyelocytic Leucemia
ASAT	Asparataminotransferase
AR	Adverse Reaction
BfArM	Bundesinstitut für Arzneimittel und Medizinprodukte
β-HCG	Human Chorionic Gonadotropin
Bili	Bilirubin
BID	twice daily
BM	bone marrow
BMI	Body Mass Index
BNP	Brain Natriuretic Peptide
BSA	Body Surface Area
CA	Competent Authority
CBC	Complete blood count
CI	Continuous infusion
CNS	central nervous system
CRA	Clinical Research Associate
CR$_{(i)(p)(m)(c)}$	Complete remission (incomplete recovery)(incomplete platelet recovery)(molecular)(cytogenetic)
CRF	Case Report Form
CRO	Clinical Research Organization
CTCAE	Common Terminology Criteria for Adverse Events
D5W	dextrose 5% solution
DLT	Dose limiting toxicity
DSUR	Development Safety Update Report
EC	Ethics Committee
eCRF	Electronic Case Report Form
ECG	echocardiography
ECOG	Eastern Cooperative Oncology Group
EDTA	Ethylenediaminetetraacetic acid
EFS	Event Free Survival
Fib	fibrinogen
FISH	Fluorescence in-situ Hybridization
FPFV	first patient first visit
GCP	Good Clinical Practice
G-CSF	Granulocyte Colony-Stimulating Factor
GI	gastrointestinal

gGT	Gamma-Glutamyl-Transpeptidase
HAM	high-dose cytarabine plus mitoxantrone
IEC	Independent Ethics Committee
INR	International Normalized Ratio (blood test)
IT	induction therapy
ITT	intent-to-treat population
IUD	intrauterine device
i.v.	intravenous
ICH	International Conference on Harmonization
ISF	Investigator Site File
KKS	Koordinierungszentrum für Klinische Studien (Center for the Coordination of Clinical Trials)
LKP	Leiterin/Leiter der klinischen Prüfung (Coordinating Investigator)
LPLV	last patient last visit
LVEF	left ventricular ejection fraction
MEC	intermediate-dose cytarabine plus mitoxantrone + etoposid
MI	myocardial infarction
MRC	Medical Research Council
MRD	Minimal Residual Disease
MTD	Maximum Tolerated Dose
MUGA	Multi Gated Acquisition
NA	not applicable
NCCN	National Comprehensive Cancer Network
ND	not done
NYHA	New York Heart Association (NYHA) Functional Classification
OS	Overall Survival
ORR	overall hematologic remission rate
PPA	per protocol analysis
p.o.	per os
PTT	Partial Thromboplastin Time
QD	Once Every Day
RCT	randomized-controlled trial
RFS	Relapse-Free Survival
RP2D	Recommended Phase 2 Dose
SAE	Serious Adverse Event
SAL	Study Alliance Leukemia
SAR	Serious Adverse Reaction
SDV	Source Data Verification
SES	Safety Evaluation Set
SmPC	summary of product characteristics
SOP	Standard Operating Procedure
SPSS	Statistical Package for the Social Sciences
SUSAR	Suspected Unexpected Serious Adverse Reaction

TAD	thioguanin + ara-C + daunorubicin
TLS	Tumor lysis syndrome
TMF	Trial Master File
Trop-T	cardiac troponin
UAR	Unexpected Adverse Reaction
V-MAC	cytarabine and mitoxantrone plus venetoclax

Involved Persons/Institutions

Sponsor:	Technische Universität Dresden 01062 Dresden Germany
Coordinating Investigator **(Leiter der Klinischen Prüfung/LKP):**	**Prof. Dr. med. Christoph Röllig** Medizinische Fakultät der TU Dresden Medizinische Klinik und Poliklinik I Fetscherstr. 74, 01307 Dresden Tel.: 0351 / 458 3775 Christoph.Roellig@ukdd.de
Medical Project Manager:	**Dr. med. Martin Wermke** Universitätsklinikum Dresden Medizinische Klinik und Poliklinik I Fetscherstr. 74, 01307 Dresden Martin.Wermke@ukdd.de **Dr. med. Theresa Kretschmann** Universitätsklinikum Dresden Medizinische Klinik und Poliklinik I Fetscherstr. 74, 01307 Dresden Theresa.Kretschmann@ukdd.de
Trial Coordination:	**Annett Haake** Medizinische Fakultät der TU Dresden Medizinische Klinik und Poliklinik I Fetscherstr. 74, 01307 Dresden Tel. 0351 / 458 3965 Annett.Haake@ukdd.de
Pharmacovigilance:	**Frank Fiebig** Medizinische Fakultät der TU Dresden Medizinische Klinik und Poliklinik I Fetscherstr. 74, 01307 Dresden
Data Management:	**Dr.rer.nat. Katharina Schmidt-Brücken** Medizinische Fakultät der TU Dresden Medizinische Klinik und Poliklinik I Fetscherstr. 74, 01307 Dresden **Koordinierungszentrum für Klinische Studien Dresden** Medizinische Fakultät Carl Gustav Carus der Technischen Universität Dresden

Monitoring:	**Dr.rer.nat. Carol El Chartouni** Bernhard-Lichtenberg-Str. 14 10407 Berlin Tel.: 0151-270 681 61 carol.chartouni@googlemail.com **Dr.rer.nat. Jana Reins** Vinetastr. 1 13189 Berlin Tel.: 0176-10424888 clinresearch@janareins.de
Biostatistician:	**Michael Kramer** Medizinische Fakultät der TU Dresden Medizinische Klinik und Poliklinik I Fetscherstr. 74, 01307 Dresden
Marketing Authorisation Holder of Study Drug:	**AbbVie Ltd.** Vanwall Road Maidenhead SL6 4UB Vereinigtes Königreich
Manufacturer of Study Drug:	**AbbVie Deutschland GmbH & Co. KG** Knollstrasse 67061 Ludwigshafen Germany
Central distribution pharmacy:	**AbbVie Deutschland GmbH & Co. KG** Knollstrasse 67061 Ludwigshafen Germany

Synopsis

Title	Phase-I/II trial for relapsed or refractory AML patients combining cytarabine and mitoxantrone with venetoclax (RELAX)
Short Title	RELAX
Target population	Patients with acute myeloid leukemia (AML) at first or second relapse after intensive chemotherapy including allogeneic stem cell transplantation or primary refractory to standard induction chemotherapy who are eligible for intensive salvage treatment
Study design	Open-label, multi-center, single-arm, phase I/II study
Sponsor	Technische Universität Dresden, 01062 Dresden
Coordinating Investigator	Prof. Dr. med. Christoph Röllig Medizinische Klinik und Poliklinik I, Universitätsklinikum TU Dresden Fetscherstr. 74, 01307 Dresden Tel. +49 351 458 13775 christoph.roellig@ukdd.de
Protocol Committee	Dr. Martin Wermke (martin.wermke@ukdd.de) Prof. Dr. med. Johannes Schetelig Dr. Moritz Middeke Medizinische Klinik und Poliklinik I, Universitätsklinikum TU Dresden Fetscherstr. 74, 01307 Dresden
Objectives of the Trial	To determine safety, tolerability, maximum tolerated dose, and recommended phase II dose of venetoclax in combination with increasing cytarabine doses plus fixed dose mitoxantrone in subjects with a relapsed or refractory AML considered fit for intensive salvage therapy. To assess the preliminary efficacy of venetoclax in combination with increasing cytarabine doses plus fixed dose mitoxantrone in subjects with a relapsed or refractory AML considered fit for intensive salvage therapy.

Background and Rationale	Patients with relapsed or refractory acute myeloid leukemia (AML) have a dismal prognosis due to moderate CR rates of around 40-55% (Steinmetz et al., Ann Hematol 1999; Karanes et al., Leuk Res 1999; Pastore et al., Ann Hematol 2003; Larsson et al., Leukemia & Lymphoma 2012; Fiegl et al., Leukemia 2014; Ahmed et al., Leuk Res 2015; Thiel et al., Ann Oncol 2015; Bergua et al., Br J Haematol 2016) and a short relapse-free and overall survival. Thus, clinical improvement by an experimental salvage regimen could be shown quite easily due to the moderate CR rates and quickly occurring events such as relapse and death under treatment with established regimens. All commonly used salvage regimens for fit AML patients worldwide are based on intermediate- to high-dose cytarabine plus mitoxantrone (HAM, MEC) or idarubicin (FLAG-Ida) (NCCN guidelines 2016; Thol et al., Blood 2015). MEC consists of cytarabine 1000 mg/m^2 on days 1-6 plus mitoxantrone 6 mg/m^2 on days 1-6 (Amadori et al., J Clin Oncol 1991), whereas HAM consists of 1000-3000 mg/m^2 BID on days 1-3 plus mitoxantrone 10 mg/m^2 on days 3-5 (Büchner et al., J Clin Oncol 2006). HAM and FLAG are common salvage regimens in Germany and Europe (Döhner et al., Blood 2010, Thol et al. 2015) whereas MEC or other intermediate-dose cytarabine combinations are used in the US (NCCN guidelines). Two randomized controlled trials failed to show a significant advantage in terms of CR or survival for fludarabine-cytarabine combinations compared to cytarabine alone (Ossenkoppele et al., Blood 2004; Fiegl et al. Leukemia 2014). In combination with low-dose cytarabine in untreated elderly AML patients unfit for intensive induction chemotherapy, the BCL-2 inhibitor venetoclax has shown promising clinical activity (Lin ASCO 2016). In addition, cytarabine may synergize with venetoclax by down-regulating Mcl-1. In a paper from 2011, Touzeau et al. showed synergy between ABT-737 and Ara-C in mantle cell lymphoma cell lines (Touzeau et al., Clin Cancer Res 2011). We hypothesize that the combination of intermediate-dose cytarabine salvage plus venetoclax will have an increased antileukemic efficacy than standard salvage alone and that efficacy signals can already be shown in a small patient number in a phase-I/II expansion cohort. Standard salvage treatments usually contain mitoxantrone or an anthracycline in combination with cytarabine. Among the most commonly used regimens MEC or HAM combine intermediate to high-dose cytarabine with mitoxantrone (see above).

	In order to prevent increased hematological toxicity after a combination of cytarabine and venetoclax, the proposed RELAX trial starts with a low-dose level of cytarabine and 400 mg venetoclax for a fixed number of days in order to allow an appropriate bone marrow recovery. Under the high safety measures of a phase-I trial, doses of cytarabine and venetoclax will be increased gradually while hematopoietic recovery will be closely monitored. The risk of excessive hematotoxicity is low because i) The targeted patient population will have a higher hematological reserve than patients in a frail setting. ii) The concept of curative relapse treatment is heavy but short cytotoxicity followed by regeneration made possibly by an almost blast-free marrow. Both issues differ significantly between patients eligible for an intensive approach and patients who receive a palliative cytoreductive treatment such as HMAs or LDAC.
Benefits and Feasibility	The following reasons explain the potential value of the trial for patient and medical science: • Medical need: patients with a very dismal prognosis – new treatments wanted for hematologists • Scientific interest: action of venetoclax in combination with intensive chemotherapy, MRD/ biomarker studies; further evaluation of potential synergies between cytarabine and venetoclax treatment • Applicability: evaluation of a venetoclax plus intermediate-dose cytarabine combination would be useful for o Future trials in relapsed/refractory AML: the dosages proposed in this RELAX trial are very similar to standard salvage regimens such as MEC or HAM or FLAG o Consolidation in first-line treatment in AML o Induction in first-line AML treatment (higher-dose cytarabine combinations are recommended treatment options for induction according to NCCN guidelines 2016 and Willemze JCO 2014) • Safety: Dose levels start with a low-dose cytarabine and 400 mg venetoclax for a fixed number of days in order to allow an appropriate bone marrow recovery, close monitoring in a 3+3 phase I

	• Rapid recruitment due to commitment of one of the two largest cooperative AML study groups in Germany (for expansion phase) and due to the chemotherapy combination being one of the most commonly used regimens in Germany (and similar to MEC in the US)
Endpoints of the Trial	Primary endpoint phase I Maximum tolerated dose of cytarabine in combination with venetoclax plus mitoxantrone in the framework of a 3+3 design Primary endpoint phase II CR/CRi rate Secondary Endpoints • Duration of remission • Cumulative incidence of relapse • Depth of remission (MRD) • Relapse-free survival • Overall survival • Early mortality (within 14 and 30 days) • Proportion of allogeneic stem cell transplantation following response • Tolerability (incidence and grade of adverse events) Exploratory endpoints • Identification of biological factors predicting CR/CRi achievement • Changes in clonal architecture of hematopoiesis during therapy
Main Inclusion Criteria	**Inclusion criteria for both escalation and expansion phase:** • Signed Informed consent • AML according to WHO-2016 criteria, excluding APL • Hematologically relapsed after first or second CR, including relapse after allogeneic stem cell transplantation • Age 18-75 years • Fit for intensive chemotherapy, defined by o ECOG 0-2, life expectancy > 3 months o Adequate hepatic and renal function (ALAT/ASAT/Bilirubin ≤2.5 x ULN; o Creatinine < 1.5 x ULN OR creatinine clearance by Cockcroft Gault formula ≥ 50 mL/min)

	• Patient is afebrile and hemodynamically stable for at least 72 hours at the time of study medication initiation. **Inclusion criteria applying for expansion phase only:** • Primary refractory after 1-2 cycles of standard induction chemotherapy (100 to 200 mg/m^2 cytarabine over 7-10 days plus anthracycline or mitoxantrone over 3 days) or hematologically relapsed after first or second CR, including relapse after allogeneic stem cell transplantation [Exact list of inclusion criteria is specified in section 4.1]
Main Exclusion Criteria	• Acute promyelocytic leukemia • CNS involvement or subjects with extramedullary disease only • Known hypersensitivity to any agent given in association with this study including cytarabine or mitoxantrone • Intended hematopoietic stem cell transplantation planned as early conditioning from aplasia without previous blood count recovery • Cumulative previous exposure to anthracyclines of >410 mg/m^2 doxorubicin equivalents • Acute GVHD ≥ grade 2, extensive chronic GVHD or requiring systemic immunosuppressive therapy • HIV infection (due to potential drug-drug interactions between antiretroviral medications and venetoclax, as well as anticipated venetoclax mechanism based lymphopenia that may potentially increase the risk of opportunistic infections) • Inability to swallow oral medications • Any malabsorption condition • Treatment with strong and moderate CYP3A inhibitors (see Appendix 1) during screening • Cardiovascular disability status of New York Heart Association Class ≥ 2. Class 2 is defined as cardiac disease in which patients are comfortable at rest but ordinary physical activity results in fatigue, palpitations, dyspnea, or anginal pain. • Chronic respiratory disease that requires continuous oxygen use. • White blood cell count > 25 × 10^9/L. Note: Hydroxyurea is permitted to meet this criterion.

<table>
<tr><td></td><td>

AML relapse treatment with any investigational or commercial drug within 14 days before enrolment. Hydroxyurea is allowed until enrolment to control peripheral WBC counts.
Substance abuse, medical, psychological, or social conditions that may interfere with the subject’s cooperation with the requirements of the trial or evaluation of the study results
Acute toxicities from any prior anti-leukemia therapy or from previous investigational drugs that have not resolved to Grade <2 per National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0
History of active or chronic infectious hepatitis unless serology demonstrates clearance of infection (Occult or prior hepatitis B virus (HBV) infection (defined as negative hepatitis B surface antigen and positive total hepatitis B core antibody) may be included if HBV DNA is undetectable, provided that they are willing to undergo monthly DNA testing. Patients who have protective titers of hepatitis B surface antibody after vaccination or prior but cured hepatitis B are eligible. Patients positive for hepatitis C virus antibody are eligible provided PCR is negative for HCV RNA)
History of clinically significant liver cirrhosis (e.g., Child-Pugh class B and C).
Pregnant or breastfeeding patients

[Exact list of exclusion criteria is specified in section 4.2]
</td></tr>
<tr><td>Trial drug</td><td>
Investigational Medicine Products:

venetoclax (ABT-199)
cytarabine (Phase I/Escalation Phase only)

</td></tr>
<tr><td>Treatment</td><td>
Phase I: Escalation phase
The induction therapy starts with the first administration of venetoclax (day 1) and patients will be admitted to the hospital for at least the first week of study treatment.
Venetoclax will be administered in increasing doses until maximum daily dose of 400 mg. The treatment schedule will be as follows:
Venetoclax ramp up
<table>
<tr><th>Day</th><th>Venetoclax daily dose</th></tr>
<tr><td>1</td><td>50 mg</td></tr>
<tr><td>2</td><td>100 mg</td></tr>
</table>
</td></tr>
</table>

3	200 mg
4 – 14 (10)	400 mg

Induction chemotherapy V-MAC

Dose level	Venetoclax target dose QD	Cytarabine daily dose	Mitoxantrone dose QD
-1	50-400 mg PO, days 1-10	100 mg/m^2 IV CI, days 4-10	10 mg/m^2 IV, days 6-8
1	50-400 mg PO, days 1-14	200 mg/m^2 IV CI, days 4-10	10 mg/m^2 IV days 6-8
2	50-400 mg PO, days 1-14	500 mg/m^2 IV BID, days 4-6	10 mg/m^2 IV days 6-8
3	50-400 mg PO, days 1-14	1000 mg/m^2 IV BID, days 4-6	10 mg/m^2 IV days 6-8

The combination of cytarabine and mitoxantrone plus venetoclax (V-MAC) will be evaluated for tolerability in a 3+3 design. In a 3+3 design, three patients will form a cohort. Each cohort will receive a higher cumulative dose of cytarabine in the predefined dose escalation steps, **starting with dose level 1** (see above). If one patient experiences a DLT, the cohort will be expanded to 6 patients. If two or more patients in a cohort experience a DLT, the next lower cytarabine dose will be defined as MTD. In case of ≥2 DLTs at the dose level 1, level -1 will be used as fallback option. In case of 0 or 1 DLT in the highest dose level, the MTD was not reached and this dose level will be defined as recommended phase-2 dose (RP2D).

The DLT evaluation period is defined as 28 days from the start of venetoclax treatment. If a patient has not recovered peripheral blood counts by day 28, the DLT evaluation period for hematologic toxicities will be extended until day 45 from start of V-MAC.

DLTs are defined as:

1) Any non-hematologic grade 3-5 toxicities related to venetoclax exposure
2) Clinical TLS (see Appendix 3)
3) Hematologic toxicities CTCAE grade 4 not recovered by day 45 in the presence of <5% bone marrow blasts (leukemia-free state):
   - neutropenia (ANC <0.5 x 10^9/L) or
   - thrombocytopenia (platelets < 25 x 10^9/L)

	The following AEs are excluded from the DLT definition: • Alopecia • Nausea and vomiting if adequately controlled by optimal supportive treatment within 7 days • Hypertension if adequately controlled by optimal treatment within 7 days • Laboratory abnormalities consistent with tumorlysis syndrome according to the modified Cairo-Bishop criteria if adequately controlled by optimal supportive treatment within 7 days. • Toxicities attributed by the investigator to a clearly identifiable, AML related, underlying illness as listed in Appendix 5. Drug-related toxicities seen more frequently than expected in prior venetoclax study may be declared a DLT for the remainder of the study after consultation between the investigator and the sponsor. Early response assessment by bone marrow aspiration will be performed in the time between day 18-21, remission control on day 28-45 (earliest on day 28 and latest on day 45 depending on regeneration of blood counts) counting from day 1 of venetoclax. **Stopping rule**: If no MTD can be determined due to high toxicity based on DLTs or if baseline toxicity data exceed expected values from historical controls or if the steering committee detects toxicity signals jeopardizing the safety of trial participants, the trial can be stopped after the completion of the phase-I part. AbbVie as the manufacturer of venetoclax shall be consulted in the process of decision making for continuation of the trial. **Phase II (expansion cohort)** The treatment dose for Phase II will be the MTD or the highest tolerable dose. At this dose level, additional 42 patients will be treated in order to provide information on the CR-rate of the combination. Patients who achieve a CR/CRp (ANC ≥1 x 10^9/L) may receive venetoclax maintenance treatment for up to one year. Dose reduction rules will be defined in case toxicities occur. Patients eligible for allogeneic stem cell transplantation with a suitable donor can proceed to transplantation and will be excluded from further study treatment.

	Maintenance Therapy: Patients eligible for maintenance treatment will receive 400 mg venetoclax QD per os as continuous treatment starting within 14 days after the remission assessment of the previous treatment phase for up to twelve 28-day cycles.
Treatment Flow Phase I and II	
Sample Size	Depending on the number of DLT at lower dose levels up to a maximum of 18 patients may be enrolled in the phase I part; For the phase-II part, in total 42 patients have to be treated at the MTD or the highest tolerable dose.
Safety	The decision to escalate to the next dose level will be taken by telephone conferences (safety calls) with participation of the investigators, pharmacovigilance and the sponsor (steering committee) on a regular basis after the DLT evaluation period of each dose level cohort. Continuous SAE monitoring will be performed in order to detect excess toxicities. In phase II, published reference values on the incidence of early mortality and toxicities ≥ grade 3 from historical cohorts will serve as external control and landmarks; excess incidences >10% will trigger extra consultation of the steering committee.
Cooperative Group	Study Alliance Leukemia (SAL) For the phase-I part of the trial, 3 sites with experienced Early Clinical Trial Units will be selected for patient recruitment in order to ensure patient safety. For the

	expansion part, additional 7 sites will be added for recruitment with an estimated overall recruitment rate of 6 patients / month.
Statistical Considerations	The phase I part will be conducted according to the enhanced algorithms of a 3+3H design (Ji et al. J Clin Oncol 2013). The phase II part will be performed as single stage study adopting the A'Hern design (A'Hern Stat Med 2001). The phase II part of the trial will test the null hypothesis that the CR/CRi rate is ≤45%. This benchmark for the CR/CRi rate w selected based on literature review of trials for this patient population. The primary efficacy analysis the CR/CRi rate will be calculated by the number of observed CR's/CRi divided by the total number of patients in the phase II full analysis set. We expect a CR/CRi rate of 65% with the combination treatment. This expectation is based on CR/CRi rates reported for the combination of venetoclax and demethylating agents in patients with de novo AML being around 70% (DiNardo ASH 2015, Pollyea ASCO 2016). The null hypothesis will be tested with the one-sample exact binomial test with a one-sided type one error probability of 5% and a power of 80%. Given 25 CR/CRis observed among the 42 patients treated at the MTD the null hypothesis will be rejected.
Anticipated Duration	Entire Trial First patient in to last patient out (months): 48 months Recruitment period (months): 24 months Follow-up period: 24 months after enrolment of last patient Follow-up period per patient: minimum 24 months after study enrolment AE reporting period: day 1 of V-MAC until 28 days after end of venetoclax treatment
Ancillary Research	Biomarkers for response to venetoclax have not yet been established for patients with AML. Response may depend on the activation of specific intracellular signaling pathways. Identification of biomarkers might help to better interpret results from clinical trials, improve the design of future trials and to identify patient groups who benefit most from this drug. Moreover, venetoclax maintenance therapy may impact on pre-malignant clonal hematopoiesis especially when the founding clones are defined by mutations in chromatin modifiers (Shlush et al., Nature 2014 and Jeziskova et al., Int J Hematol 2015).

Therefore, we will set out to study changes in the clonal composition of hematopoiesis in patients who achieved a remission.

We will apply targeted sequencing of genes implicated in AML pathogenesis, chemotherapy resistance and clonal hematopoiesis and perform RNA expression profiling for selected genes. Peripheral blood/bone marrow samples will be collected at screening, in remission and at end of cycle 2, 6 and 12 during maintenance therapy and at relapse/progression of AML according to the following schedule:

Timepoint / required material	Screening	CR/ CRi	during maintenance therapy			Relapse / Progress
			End of cycle 2	End of cycle 6	End of cycle 12 = EOT	
Targeted DNA-sequencing and RNA-expression profiling of purified bone marrow (BM) blasts - 5 ml BM in EDTA Tube - 3 ml heparinized BM	x	x				x
Targeted DNA-sequencing of peripheral blood (pB) - 9 ml pB in EDTA Tube	x	x	x	x	x	x

Goals of the ancillary research project are:

1) To determine biomarkers for response and mechanisms of resistance to the combination of venetoclax plus ARAC-based chemotherapy
2) To display the course of minimal residual disease
3) To test if changes the clonal composition of hematopoiesis of patients occur during maintenance therapy

1 Introduction

1.1 Background relapsed/refractory AML

Patients with relapsed or refractory acute myeloid leukemia (AML) have a dismal prognosis due to moderate CR rates of around 40-55% (Steinmetz et al., Ann Hematol 1999; Karanes et al., Leuk Res 1999; Pastore et al., Ann Hematol 2003; Larsson et al., Leukemia & Lymphoma 2012; Fiegl et al., Leukemia 2014; Ahmed et al., Leuk Res 2015; Thiel et al., Ann Oncol 2015; Bergua et al., Br J Haematol 2016) and a short relapse-free and overall survival.

1.2 Treatment regimens

All commonly used salvage regimens for fit AML patients worldwide are based on intermediate- to high-dose cytarabine plus mitoxantrone (HAM, MEC) or idarubicin (FLAG-Ida) (NCCN guidelines 2016; Thol et al., Blood 2015). MEC consists of cytarabine 1000 mg/m^2 on days 1-6 plus mitoxantrone 6 mg/m^2 on days 1-6 (Amadori et al., J Clin Oncol 1991), whereas HAM consists of 1000-3000 mg/m^2 BID on days 1-3 plus mitoxantrone 10 mg/m^2 on days 3-5 (Büchner et al., J Clin Oncol 2006). HAM and FLAG are common salvage regimens in Germany and Europe (Döhner et al., Blood 2010, Thol et al. 2015) whereas MEC or other intermediate dose cytarabine combinations are used in the US (NCCN guidelines). Two randomized controlled trials failed to show a significant advantage in terms of CR or survival for fludarabine-cytarabine combinations compared to cytarabine alone (Ossenkoppele et al., Blood 2004; Fiegl et al. Leukemia 2014).

Clinical improvement by an experimental salvage regimen could be shown quite easily due to the moderate CR rates and quickly occurring events such as relapse and death under treatment with established regimens.

1.3 Venetoclax

Venetoclax (also referred to as ABT-199 and GDC-0199) is a novel, orally bioavailable, smallmolecule B-cell lymphoma-2 (BCL-2) family inhibitor in the biarylacylsulfonamide chemical class. Venetoclax binds with high affinity (inhibition constant [K i] < 0.010 nM) to antiapoptotic protein BCL-2 and with lower affinity to other antiapoptotic Bcl -2 family proteins, like Bcl-XL and Bcl-w (> 4,000-fold and > 2,000- to > 20,000-fold lower affinity than to BCL-2, respectively).
Antiapoptotic BCL-2 family members are associated with tumor initiation, disease progression, and chemotherapy resistance, as well as autoimmunity. Overexpression of BCL-2 is a major contributor to the pathogenesis of some lymphoid malignancies; antagonism of the action of these proteins may enhance response to therapy and overcome resistance, and thus, these proteins are compelling targets for anti-tumor therapy (Fesik, Nat Rev Cancer 2005).

1.3.1 Summary of data

Venetoclax is a novel, orally bioavailable, small molecule BCL-2 family inhibitor in the biarylacylsulfonamide chemical class. Venetoclax binds with high affinity (Ki < 0.010 nM) to antiapoptotic protein BCL-2 and with lower affinity to other antiapoptotic BCL-2 family proteins, like Bcl-XL and Bcl-w (> 4,000-fold and > 2,000-fold to > 20,000-fold lower affinity than to BCL-2, respectively). Venetoclax is projected to have a different safety profile than dual BCL-2/Bcl-XL inhibitors. Survival of platelets depends on BCL-XL and thrombocytopenia is therefore a major DLT caused by inhibition of BCL -XL in the clinic. Venetoclax is expected to yield an improved therapeutic index by maintaining efficacy against tumor cells while avoiding dose-limiting thrombocytopenia.

Nonclinical and clinical data available with venetoclax are summarized below.

Oncology non-clinical pharmacology: In vitro, venetoclax demonstrated cell killing activity against patient-derived CLL and AML cells and a variety of lymphoma and leukemia cell lines, including B-cell FLs, MCLs, DLBCLs, AMLs, and MM. Venetoclax was especially potent against NHL cell lines expressing high levels of BCL-2. Venetoclax inhibits subcutaneous xenograft growth of human tumor cell lines derived from ALL, NHL, and AML, and is highly efficacious using various doses and combined with other regimens. The drug is also active in a model of disseminated ALL and AML.

Non-clinical safety pharmacology: Venetoclax was tested in a battery of safety pharmacology assays and produced no effects in CNS/neurobehavioral, or respiratory studies in mice at oral doses up to 600 mg/kg. In dogs, mild reductions in cardiac contractility and cardiac output were observed at plasma concentrations of ≥ 16 µg/mL; concentrations greater than the concentration of venetoclax in humans (average Cmax = 6.09 µg/mL at the 1200 mg dose). However, no effects on blood pressure, heart rate, or ECG parameters were observed in dogs at a maximum drug concentration of 46 µg/mL.

Non-clinical product metabolism: Venetoclax exhibited moderate permeability in the Caco-2 assay. In rats, venetoclax was widely distributed into liver, kidneys, spleen, heart, lungs, small intestine, and white fat, but was poorly distributed in testes, brain, muscle, bone, and pigmented tissues. Venetoclax showed moderate in vitro metabolic stability in hepatic systems across species tested, with the exception of dog hepatocytes, where stability was low to moderate. In rats, 14.3% of the dose was recovered as parent drug after 48 hours post-dose, while 76.9% of the dose was excreted as metabolites in bile. Profiles in bile indicated that metabolism was the major clearance mechanism, while biliary excretion of parent drug played a secondary role in drug elimination.

Biotransformation of [3H] venetoclax proceeded via a combination of oxidation and conjugation. Venetoclax ispredominately metabolized by CYP3A4 in vitro, thus CYP3A4 inhibitors or inducers are expected to cause changes in venetoclax exposure. Clinical studies have supported the in vitro observations for venetoclax as a sensitive substrate of CYP3A4: > 5-fold increase in AUC when co-dosed with ketoconazole, and > 50% decrease in AUC when co-dosed with rifampin. At the 400 mg QD dose venetoclax is not predicted to be perpetrator of the major CYP enzymes, but venetoclax may weakly inhibit UGT1A1. Venetoclax is substrate for the efflux transporters P-gp and BCRP, and inhibitors or inducers of these transporters are expected to cause change in the exposure of venetoclax. Venetoclax is P-gp and BCRP inhibitor and may interact with substrates for these transporters. Venetoclax may inhibit OATP1B1 and cause weak interaction with drugs that are substrates of this transporter.

Non-clinical toxicology: The primary toxicities associated with repeat-dose administration of venetoclax were effects on the hematologic system (decreased lymphocytes and RBC mass in mice, rats and dogs), the male reproductive system (testicular germ cell depletion in dogs) and embryofetal toxicity in mice. Other noteworthy findings were epithelial single cell necrosis in multiple tissues and hair coat color change, both in dogs. For lymphocyte reductions, B cells were the most sensitive lymphocyte subtype based on the magnitude of decrease and/or the length of time required for recovery. Decreases of lymphocytes were reversible or partially reversible; decreases in erythrocytes were reversible. Venetoclax resulted in increased post-implantation loss and decreased fetal body weights in the mouse embryofetal development study at the highest dosage administered (150 mg/kg/day); the NOAEL was defined at the mid-dose of 50 mg/kg/day. Venetoclax was not teratogenic, and there were no other effects on development or fertility. Venetoclax was also negative in an in vivo phototoxicity assessment. In vitro genotoxicity studies (Ames and chromosome aberration assays) conducted on a major human metabolite.

Clinical pharmacokinetics: The venetoclax formulation currently used in clinical studies is a tablet formulation with strengths of 10, 50, and 100 mg. Following multiple-dose administration, the maximum plasma concentration of venetoclax was attained 5 to 8 hours after dosing. The harmonic mean terminal half-life (t1/2) ranged from 17 to 41 hours following a single oral dose of venetoclax. In subjects with CLL, venetoclax showed minimal accumulation, and steady-state AUC increased proportionally over the dose range of 150 to 800 mg. Venetoclax has been administered with food in all clinical studies, as food increased the bioavailability of venetoclax by approximately 3 - to 5-fold. Venetoclax is highly bound to plasma proteins with unbound fraction (fu) < 0.01, and it is primarily eliminated as metabolites in feces with negligible renal elimination (< 0.1%). Additionally, based on the population pharmacokinetic analysis, age, sex, race, weight, mild or moderate hepatic and renal impairment do not have an effect on venetoclax clearance.

Clinical safety and efficacy: A total of 1662 subjects have been exposed to at least 1 dose of venetoclax in the oncology and immunology development programs. A total of 1498 oncology subjects had data available in AbbVie and Genentech/Roche studies as of 28 November 2015. Of these 1498, 935 subjects had CLL/SLL, 346 subjects had NHL, 115 subjects had MM, 102 had AML; an additional 66 subjects were healthy volunteers. A total of 564 oncology subjects received the drug as monotherapy and 933 received the drug in combination with other therapies (rituximab, obinutuzumab, R-CHOP, G-CHOP, BR, BG, bortezomib plus dexamethasone, azacitidine, decitabine, and cytarabine).

Important identified risks are TLS and neutropenia, particularly in CLL indication. Infection is a potential risk. Other adverse events commonly observed with venetoclax include nausea, diarrhea, and other hematological effects (including, anemia, thrombocytopenia, and lymphopenia). Decreased spermatogenesis has been observed in non-clinical studies with dogs, effect in humans is unknown. In addition, as venetoclax is being evaluated in subjects with relapsed/refractory disease who had previously been treated with various cytotoxic agents, second primary malignancies are closely monitored.

In subjects with AML, the ORR was 19% for venetoclax monotherapy (Study M14 -212) and was 77% for venetoclax in combination with azacitidine or decitabine (Study M14 -358).

1.3.2 Guidance for investigators

On the basis of non-clinical data and previous experience with BCL-2 inhibitors, as well as the available preliminary data in the venetoclax clinical oncology program, the following guidance is provided for the investigator.

Contraindications

Concomitant use of venetoclax with strong and moderate CYP3A inhibitors at initiation and during the dose titration phase is contraindicated (section 5.5).

Warning and Precautions

The following sections are a summary of the identified and potential risks when dosing with venetoclax, and the specific safety measures that need to be taken.

Tumor Lysis Syndrome (Oncology Studies)

Tumor lysis syndrome (TLS) is an important identified risk for venetoclax in oncology studies, especially in CLL. As a result of on-target effects, the potential for TLS was identified early in the program. The available data suggest that in non-CLL subjects the risk of TLS is low. Since the

data set is small, the risk of TLS is being closely monitored in non-CLL indications. In general, before initiating venetoclax, subjects risk for developing TLS should be assessed. Prophylaxis with hydration and uric-acid reducing agents is recommended. Clinical chemistries should be corrected. Monitor with clinical chemistries and manage promptly, as clinically indicated. Please refer to detailed guidance for TLS management in section 5.4.5.

Neutropenia

Neutropenia is an important identified risk for venetoclax, specifically in CLL. Clinical data from the oncology studies suggest that the neutropenia adverse events are observed among subjects who receive venetoclax as a single agent or in combination with other therapeutic agents, with slightly higher frequency observed in some combination studies.

Infections

Infections have been reported in the oncology clinical studies; however, these events are confounded by the underlying disease, comorbidities, and other immunosuppressive medications. To date, no clear relationship has been noted between serious infectious events and neutropenia. The types of infectious events observed generally have been consistent with those anticipated in the elderly population of heavily pretreated subjects with hematologic malignancies and are similar across all indications.

Other Hematological Effects

Anemia has been reported in the oncology studies with slightly higher frequency in some studies in which venetoclax is combined with other chemotherapeutic agents; however, most of the events were non-serious and confounded by disease factors and prior therapies. The dataset in non-CLL indications is small.

Thrombocytopenia adverse events have been reported in the oncology studies, with slightly higher frequency in studies in which venetoclax is combined with other chemotherapeutic agents. However, most of the events were non-serious and assessment of these events is confounded by the subjects' underlying disease state, prior therapies and preexisting thrombocytopenia, including autoimmune thrombocytopenia in several subjects. The dataset in non-CLL indications is small.

Lymphopenia has been observed in preclinical studies. While opportunistic infections have been reported in the clinical program, data is confounded by subjects underlying disease and prior therapies. In the oncology studies, anti-infective prophylaxis should be implemented as clinically indicated, including appropriate prophylaxis for viral, fungal, bacterial, or Pneumocystis carinii pneumonia infections.

Reproductive System Effects

Based on nonclinical studies, there is a potential for decreased spermatogenesis. Non-reversible depletion of testicular germ cells has been observed in dogs at all doses tested after 4 weeks of dosing. In the oncology studies, male subjects should be instructed to consider sperm banking before treatment with venetoclax if they are considering preservation of fertility. Male subjects are excluded from the initial SLE studies and healthy volunteer studies.

Treatment-Emergent Malignancies (Second Primary Malignancies)

Events of second primary malignancies have been reported across the oncology program. No pattern has been observed. As venetoclax is being evaluated in subjects with relapsed/refractory disease who had previously been treated with various cytotoxic agents, second primary malignancies are closely monitored.

Food Effect

Administration with a low-fat meal increased venetoclax exposure by approximately 3.4-fold and administration with a high-fat meal increased venetoclax exposure by 5.1- to 5.3-fold compared to fasting conditions. Venetoclax should be administered with a meal.

Vulnerable Patient Populations

Pregnancy

Although no potential risks have been identified in nonclinical studies, the effect of BCL-2 inhibition on pregnancy has not been fully characterized. Venetoclax resulted in increased post-implantation loss, and decreased fetal body weights were observed in the mouse embryofetal development study at the highest dosage administered. Venetoclax was not teratogenic, and there were no other effects on development or fertility. Two pregnancies have been reported in the program, including one pregnancy of a partner. Venetoclax should not be administered to pregnant women. Female subjects enrolled in clinical studies with single agent venetoclax must be surgically sterile, postmenopausal (for at least 1 year), or have negative results for a pregnancy test. Female subjects enrolled in clinical studies with venetoclax must agree to use contraception from initial study drug administration until 30 days after the last dose of study drug. Venetoclax must be discontinued if a female subject becomes pregnant (i.e. subject will be excluded from the study).

Male subjects enrolled in clinical studies with single agent venetoclax must agree to use contraception from initial study drug administration until 30 days after the last dose of study drug.

Nursing Mothers

It is not known whether venetoclax is excreted in human milk. Hence, until further data becomes available, venetoclax should not be administered to nursing mothers.

Children

Safety and effectiveness in pediatric patients under 18 years of age have not been established; therefore, venetoclax should not be administered to this patient population.

Geriatric Patients

Venetoclax has been administered to elderly subjects (> 65 years) across the oncology program. The available data do not demonstrate any additional safety concerns when administrating venetoclax in the elderly population.

Concomitant Use with Other Medications

During Phase I/Escalation part, strong and moderate CYP3A inhibitors and inducers (see Appendix 1) are prohibited.
If a patient critically needs e.g. anti-fungal treatment **after** the end of the venetoclax treatment period, that should be discussed with the coordinating investigator and the decision should be made based on the medical/clinical need.

For Phase II/Expansion part, the concomitant use of strong and moderate CYP3A inhibitors is allowed, but the dose of venetoclax should be reduced as described in section 5.5.

Venetoclax should be administered using caution with substrates or inhibitors of P-gp, BCRP, or substrates of OATP1B1/B3. If venetoclax is co-administered with warfarin, the international normalized ratio (INR) should be monitored closely.

Live-virus vaccines should not be given within 28 days prior to the initiation of study treatment, at any time during study treatment, or in the 30 days following last dose of study treatment.

Overdose

Dosing with venetoclax may vary with indication, with the maximum evaluated dose for venetoclax being 1200 mg. In any case in this trial the maximum daily dose of 400mg should not be exceeded.

In the event of the adverse event of overdosing, appropriate supportive treatment should be initiated according to the subject's clinical signs and symptoms.

1.4 Rational of study and risk-benefit assessment

In combination with low-dose cytarabine, the bcl2 inhibitor venetoclax has shown promising clinical activity with a CR+CRi rate of 54% in a frail elderly patient population receiving initial treatment for newly diagnosed AML (Wei et al., ASH 2016). In addition, cytarabine may synergize with venetoclax by down-regulating Mcl-1. In a paper from 2011, Touzeau et al.b showed synergy between ABT-737 and Ara-C in mantle cell lymphoma cell lines (Touzeau et al., Clin Cancer Res 2011). We hypothesize that the combination of intermediate-dose cytarabine salvage plus venetoclax will have an increased antileukemic efficacy than standard salvage alone and that efficacy signals can already be shown in a small patient number in a phase-I/II expansion cohort. Based on historical data showing around 20% CRs in frail patients treated with low-dose cytarabine alone (Burnett et al., Cancer 2007), the combination with venetoclax results in an increase by 30%. Standard intensive relapse treatments produce CR rates of around 40-55% (Steinmetz et al., Ann Hematol 1999; Karanes et al., Leuk Res 1999; Pastore et al., Ann Hematol 2003; Larsson et al., Leukemia & Lymphoma 2012; Fiegl et al., Leukemia 2014; Ahmed et al., Leuk Res 2015; Thiel et al., Ann Oncol 2015; Bergua et al., Br J Haematol 2016). Assuming a similar beneficial effect of venetoclax in this setting, we assume a CR rate of 65% for the combination treatment of intermediate-dose cytarabine, mitoxantrone and venetoclax. The phase II part will be performed as single stage study adopting the A'Hern design (A'Hern Stat Med 2001) with a sample size of 42 patients (for detailed sample size calculation see section 8.2.1).

In order to prevent increased hematological toxicity after a combination of cytarabine and venetoclax, the proposed RELAX trial starts with a low-dose level of cytarabine and 400mg venetoclax for a fixed number of days in order to allow an appropriate bone marrow recovery. Under the high safety measures of a phase-I trial, doses of cytarabine and venetoclax will be increased gradually while hematopoietic recovery will be closely monitored. The risk of excessive hematotoxicity is low because:

- The targeted patient population will have a higher hematological reserve than patients in a frail setting.
- The concept of curative relapse treatment is heavy but short cytotoxicity followed by regeneration made possibly by an almost blast-free marrow. Both issues differ significantly between patients eligible for an intensive approach and patients who receive a palliative cytoreductive treatment such as HMAs or LDAC.

Several important reasons justify the set-up of the RELAX trial:

- Medical need: patients with a very dismal prognosis – new treatments wanted for hematologists
- Scientific interest: action of venetoclax in combination with intensive chemotherapy, MRD/ biomarker studies; further evaluation of potential synergies between cytarabine and venetoclax
- Applicability: evaluation of a venetoclax plus intermediate-dose cytarabine combination would be useful for
  - Future trials in relapsed/refractory AML: the dosages proposed in this RELAX trial are very similar to standard salvage regimens such as MEC or HAM or FLAG
  - Consolidation in first-line treatment in AML
  - Induction in first-line AML treatment (higher-dose cytarabine combinations are recommended treatment options for induction according to NCCN guidelines 2016 and Willemze JCO 2014)
- Evaluation of minimal residual disease/ clonal hematopoiesis during a maintenance phase will help to explore the potential of venetoclax continuous dosing in AML and define possible future application modes in AML such as maintenance or non-chemo-based consolidation
- Safety: Dose levels starts with a low-dose cytarabine and 400 mg venetoclax for a fixed number of days in order to allow an appropriate bone marrow recovery, close monitoring in a 3+3 phase I
- Feasibility: limited patient number with potentially high clinical impact due to low CR rates and short survival in relapsed/refractory AML, rapid recruitment due to the chemotherapy combination cytarabine plus mitoxantrone (HAM) being one of the most commonly used regimens in Germany (and similar to the combination of cytarabine, mitoxantrone and etoposide [MEC] in the US)

The study population comprises patients with relapsed AML who are fit for intensive salvage treatment, i.e. for treatment with a curative intention. In this patient group, the primary treatment goal is the induction of a leukemia-free state or complete remission as a prerequisite for post-remission treatment and long-term survival. There are two options for post-remission treatment: All patients in remission, with a sufficient medical condition and an available matched donor should proceed to allogeneic SCT whereas all other patients should receive intensive cytarabine-based consolidation treatment. The ultimate goal of this trial is to improve the efficacy of intensive salvage treatment by adding venetoclax as a new promising compound to a standard salvage regimen (cytarabine plus mitoxantrone). A more effective salvage regimen would increase the chances of relapsed patients to achieve an AML remission, which is a prerequisite for long-term survival.

Since venetoclax has not been combined with higher doses of cytarabine plus mitoxantrone yet, this trial intents to evaluate the MTD of this combination and to find the recommended dose for the expansion cohort and subsequent randomized trials.

In an ongoing phase-I trial, venetoclax in a dose of 600 mg over 14 days has been combined with 100 mg/m^2 cytarabine over five days plus idarubicin 12 mg/m^2 over two days in elderly AML patients with no unexpected toxicity. Based on the results of this trial, the combination of 600 mg venetoclax over 14 days in combination with 5+2 chemotherapy is considered tolerable and safe (Wei et al., 2018). The venetoclax dose in the RELAX trial is lower (400 mg). It is therefore considered safe to combine the lower venetoclax dose with 200 mg/m^2 cytarabine in the first dose level.

In order to avoid undertreatment of patients in the low cytarabine dose levels in the dose escalation part of the trial, only patients relapsing >90 days after previous cytarabine chemotherapy will be allowed into this part of the trial. This is because >90 days after last cytarabine dose, the disease is again considered sensitive to cytarabine, justifying the treatment of patients even with lower cytarabine doses. Patients refractory to standard-dose cytarabine may not be responsive to lower cytarabine doses as given in the first dose levels of this trial and are therefore excluded from enrolment in the escalation part, but not in the expansion part where higher cytarabine doses will be administered.

The benefit for participating patients will be the higher probability to achieve a remission as suggested by 70% remission rates in frail patients with primary AML (Wei et al, 2016). The addition of fixed-dose mitoxantrone ensures a response to treatment even in the initial dose levels. The main risks result from unknown toxicities arising from the combination. However, the known toxicities of venetoclax are hematological in nature. Given that the primary aim of any salvage regimen is to induce a significant blast reduction or morphologic leukemia free state as a prerequisite for the success of further consolidation treatments aiming at inducing a long-term CR, treatment related cytopenias seem to be less important in the setting of relapsed/refractory AML than in primary AML treatment and are intended in AML relapse treatment in order to eradicate the relapsing and supposedly resistant leukemic AML clone. As a result of these considerations, possible benefits of study treatment will outweigh potential risks. Additionally, risks will be minimized by toxicity monitoring and pharmacovigilance measures.

2 Study objectives and purpose

The primary objective of this study is to

- Determine the safety, tolerability and maximum tolerated dose of cytarabine in combination with fixed dose venetoclax plus fixed dose mitoxantrone in subjects with a relapsed or refractory AML considered fit for intensive salvage therapy.

The secondary objective of this study is to

- Assess the preliminary efficacy of venetoclax in combination with increasing cytarabine doses plus fixed dose mitoxantrone in subjects with a relapsed or refractory AML considered fit for intensive salvage therapy.

Exploratory objectives of this study are to

- Identification of biological factors predicting CR/CRi achievement
- Changes in clonal architecture of hematopoiesis during therapy

3 Study design

3.1 Design overview

This is an open-label Phase I dose-escalation study of oral venetoclax in combination with increasing cytarabine doses plus mitoxantrone to define the safety profile and MTD of cytarabine in subjects with a histologically or cytologically confirmed acute myeloid leukemia who are refractory or suffered a relapse. This study will be conducted at multiple centers in Germany.

This study will investigate the combination of a fixed maximum venetoclax dose with increasing cytarabine doses plus mitoxantrone in a fixed dose. The venetoclax dose of 400 mg will be reached by a ramp up over 4 days (see Table 2). Parallel chemotherapy with cytarabine will start on day 4. This dose escalation part will enroll a maximum number of 18 patients.

The decision to escalate to the next dose level will be taken by telephone conferences (safety calls) with participation of the investigators, pharmacovigilance and the sponsor (steering committee) on a regular basis.
AbbVie as the manufacturer of venetoclax shall be consulted in the process of decision making for continuation of the trial.

The dose-escalation part may be followed by an expansion part with enrollment of 42 patients. The decision to proceed to the expansion part will be taken and documented in consultation within

the steering committee after consideration of all available study data during the dose escalation part of the study. These results of data to safety and tolerability of the therapy during the escalation part will be submitted as a substantial amendment to the competent authority and ethics committee for approval the recommended phase-2 dose (RP2D).Patients who achieve a CR/CRp (ANC ≥1 x $10^{9/}$L) may receive venetoclax maintenance treatment at 400 mg QD p.o. continuously for up to one year. Dose reduction rules will be defined in case toxicities occur. Patients eligible for allogeneic stem cell transplantation with a suitable donor can proceed to transplantation and will be excluded from further study therapy but will further be observed during follow-up period.

3.2 Definition and determination of the maximum tolerated dose

The combination of cytarabine and mitoxantrone plus venetoclax will be evaluated for tolerability in a 3+3 design. In a 3+3 design, three patients will form a cohort. Each cohort will receive a higher cumulative dose of cytarabine in the predefined dose escalation steps (see Table 1) in combination with a fixed dose of mitoxantrone and venetoclax. If one patient in a cohort experiences a DLT, the cohort will be expanded to 6 patients. If 0/3 or 1/6 patients in a cohort experience a DLT, the next dose escalation cohort will be opened. If two or more patients in a cohort experience a DLT, the next lower cytarabine dose will be defined as MTD. In case of ≥2 DLTs at the dose level 1, level -1 will be used as fallback option. In case of 0 or 1 DLT in the highest dose level, the MTD was not reached and this dose level will be defined as recommended phase-2 dose (RP2D).

The DLT evaluation period is defined as the first 28 days from the start of venetoclax treatment. If a patient has not recovered peripheral blood counts by day 28, the DLT evaluation period for hematologic toxicities will be extended until day 45 from start of V-MAC.

Note: If a DLT of TLS occurs during the lead-in period, it may affect dosing decisions in the ramp up period.

A DLT is defined as any of the following occurring events of a dose level and regarded to be probably or definitely related to venetoclax. The assessment is based on the question whether there was a “reasonable causal relationship” to venetoclax. Possible answers are “possible” or “unlikely”. An assessment of “unlikely” would include the existence of a clear alternative explanation, e.g. mechanical bleeding at surgical site or non-plausibility, e.g. the subject is struck by an automobile when there is no indication that the drug caused disorientation that may have caused the event.

Certain adverse events are likely to occur in the study population (AML) independent of drug exposure. Such events include known consequences of the underlying disease under

investigation (e.g. symptoms, disease progression). These events are listed in Appendix 5. If not mentioned in Appendix, events with no reasonable causal relationship to venetoclax will not be considered as DLT. On the other hand, unlikely related events with unusually high occurrence during the trial could be considered DLT if agreed by the steering committee.

CTCAE v5.0 will be used to assess toxicities.

DLTs are defined as:

1) Any non-hematologic grade 3-5 toxicities related to venetoclax exposure
2) Clinical TLS (see Appendix 3)
3) Hematologic toxicities CTCAE grade 4 not recovered by day 45 in the presence of <5% bone marrow blasts (leukemia-free state):
    - neutropenia (ANC $<0.5 \times 10^9$/L) or
    - thrombocytopenia (platelets $< 25 \times 10^9$/L)

The following AEs are excluded from the DLT definition:

- Alopecia
- Nausea and vomiting if adequately controlled by optimal supportive treatment within 7 days
- Hypertension if adequately controlled by optimal treatment within 7 days
- Laboratory abnormalities consistent with tumorlysis syndrome according to the modified Cairo-Bishop criteria if adequately controlled by optimal supportive treatment within 7 days.
- Toxicities attributed by the investigator to a clearly identifiable, AML related, underlying illness as listed in Appendix 5.

Drug-related toxicities seen more frequently than in the prior venetoclax study may be declared a DLT for the remainder of the study after consultation between the steering committee and the sponsor.

Safety monitoring will occur by telephone conferences (safety calls) of the steering committee as soon as the last patient of the respective cohort has passed the complete DLT-evaluation period (at latest on day 45 from start of V-MAC) and all relevant clinical safety data are available. During this safety calls will be discussed and decided about the dose level which continues the trial.

Stopping rule: If no MTD can be determined due to high toxicity based on DLTs or if baseline toxicity data exceed expected values from historical controls or if the steering committee detects

toxicity signals jeopardizing the safety of trial participants, the trial can be stopped after the completion of the phase-I part.

3.2.1 Target dose for DLT evaluation

To ensure the required number of evaluable subjects per cohort, patients have to have received at minimum the following of study treatment. This will be defined as ≥ 3800 mg venetoclax, ≥ 2 days of mitoxantrone, and ≥ 6 days of cytarabine in cohort 1 and ≥ 3800 mg venetoclax, ≥ 2 days of mitoxantrone, and ≥ 2.5 days of cytarabine in cohorts 2+3. The dosage of mitoxantrone and cytarabine has to be according to protocol.

3.3 Expansion cohort

The treatment dose of Phase II will be the MTD or the highest tolerable dose. At this dose level additional 42 patients will be treated in order to provide information on the CR-rate of the combination.

In phase II, published reference values on the incidence of early mortality and toxicities ≥ grade 3 from historical cohorts will serve as external control and landmarks; excess incidences >10% will trigger extra consultation of the steering committee.

Patients in CR/CRp (ANC ≥1 x 10^9/L) may receive venetoclax maintenance treatment at a maximum dose of 400 mg QD p.o. continuously for up to twelve 28-day cycles. Dose reduction rules will be defined in case toxicities occur. Patients eligible for allogeneic stem cell transplantation with a suitable donor can proceed to transplantation and will be excluded from further study treatment.

3.4 Leukemia response evaluation and treatment continuation

Early response assessment by bone marrow aspiration will be performed in the time between day 18-21 (only cytomorphology), remission control on day 28-45 (earliest on day 28 and latest on day 45 depending on regeneration of blood counts) counting from day 1 of venetoclax (indicated in the visit schedule in section 6.1/Table 9).

Patients in CR/CRp (ANC ≥1 x 10^9/L) may receive venetoclax maintenance treatment at a maximum dose of 400 mg QD p.o. continuously for up to twelve 28-day cycles, if they do not have any other post-remission treatment option (e.g. allo/auto-TX, consolidation chemotherapy etc.). Assessments for safety and efficacy during maintenance therapy see Table 10.

Further treatment of the patients other than venetoclax maintenance will be outside this clinical trial at the physicians' discretion.

3.5 Endpoints (primary, secondary)

Primary endpoint phase I (dose escalation)

Maximum tolerated dose of cytarabine in combination with venetoclax plus mitoxantrone in the framework of a 3+3 design

Primary endpoint phase II (expansion)

CR/CRi rate

Secondary Endpoints

- Duration of remission
- Cumulative incidence of relapse
- Depth of remission (MRD)
- Relapse-free survival
- Overall survival
- Early mortality (within 14 and 30 days)
- Proportion of allogeneic stem cell transplantation following response
- Tolerability (incidence and grade of adverse events)

Exploratory Endpoints

- Identification of biological factors predicting CR/CRi achievement
- Changes in clonal architecture of hematopoiesis during therapy

3.6 Number of study centers

For the phase-I part of the trial, 3 sites with experienced Early Clinical Trial Units will be selected for patient recruitment in order to ensure patient safety.

For the expansion part, additional 7 sites will be added for recruitment with an estimated overall recruitment rate of 6 patients/ month.

3.7 Patient assignment and expected duration of subject participation

Each subject who signs a consent form for the study and undergoes any screening procedure will be assigned a unique 5-digit number during the screening period. The first 3 digits are site specific; the remaining 2 digits are subject specific and allocated in a sequential order of subjects starting the screening period. This screening number will be entered into the eCRF regardless of whether

the subject is actually treated with study drug. Subjects not treated will be considered screening failures. The reason for screening failure should be recorded in the eCRF.

Patients are expected to be on study for at least 28 days from first dose of venetoclax and until:

- Phase I – minimum until the end of DLT evaluation period (day 45 from start venetoclax), if no maintenance therapy is planned
- Phase II – minimum until remission assessment (day 45 from start venetoclax), if no maintenance therapy is planned
- If maintenance therapy is started – until 28 days after the last dose of venetoclax

For premature discontinuation see withdrawal of subjects in section 4.4.1.

4 Study population

Screening procedures must have been performed within 14 days before the first dose of study drug.

4.1 Inclusion criteria

Inclusion criteria for both escalation and expansion phase:

- Ability to understand and the willingness to sign a written informed consent. A signed informed consent must be obtained before screening.
- AML according to WHO-2016 criteria, excluding acute promyelocytic leukemia
- Relapsed from first or second CR after 1-2 cycles of standard induction chemotherapy (which must have included cytarabine with an anthracycline or anthracenedione), including relapse after allogeneic stem cell transplantation (dose escalation and expansion part)
- Age 18-75 years
- Fit for intensive chemotherapy, defined by
  - ECOG 0-2, life expectancy > 3months
  - Adequate hepatic function: ALAT/ASAT/Bilirubin ≤2.5 x ULN*

    * unless considered due to leukemic organ involvement

    Note: Subjects with Gilbert's Syndrome may have a bilirubin > 2.5 × ULN per discussion between the investigator and Coordinating investigator.
  - Adequate renal function assessed by serum creatinine ≤ 1.5x ULN OR creatinine clearance (by Cockcroft Gault formula) ≥ 50 mL/min

- Patient is afebrile and hemodynamically stable for at least 72 hours at the time of study medication initiation.
- Male subjects must agree to refrain from unprotected sex and sperm donation from time point of signing the informed consent until 30 days after the last dose of study drug.
- Women must fulfill at least one of the following criteria in order to be eligible for trial inclusion:
    - Post-menopausal (12 months of natural amenorrhea or 6 months of amenorrhea with Serum FSH > 40 U/ml)
    - Postoperative (i.e. 6 weeks) after bilateral ovariectomy with or without hysterectomy
    - Women of childbearing potential must have a negative serum pregnancy test performed within 7 days before the first dose of study drug.
    - Continuous and correct application of a contraception method with a Pearl Index of <1% (e.g. implants, depots, oral contraceptives, intrauterine device – IUD) from time point of signing the informed consent until 30 days after the last dose of study drug.

      Note: At present, it is not known whether the effectiveness of hormonal contraceptives is reduced by venetoclax. For this reason, women should use a barrier method in addition to hormonal contraceptive methods.
    - Sexual abstinence
    - Vasectomy of the sexual partner

Inclusion criteria applying for expansion phase (Phase II) only:

- Primary refractory after 1-2 cycles of standard induction chemotherapy (100 to 200 mg/m2 cytarabine over 7-10 days plus anthracycline or mitoxantrone over 3 days) or relapsed from first or second CR after 1-2 cycles of standard induction chemotherapy (which must have included cytarabine with an anthracycline or anthracenedione), including relapse after allogeneic stem cell transplantation

4.2 Exclusion criteria

- Acute promyelocytic leukemia (AML M3)
- CNS involvement or subjects with extramedullary disease only
- Known hypersensitivity to excipients of the preparation or any agent given in association with this study including cytarabine or mitoxantrone
- Intended hematopoietic stem cell transplantation planned as early conditioning from aplasia without previous blood count recovery

- Cumulative previous exposure to anthracyclines of >410 mg/m^2 doxorubicin equivalents
- Acute GVHD ≥ grade 2, extensive chronic GVHD or requiring systemic immunosuppressive therapy within 2 weeks prior to start of study treatment
- HIV infection (due to potential drug-drug interactions between antiretroviral medications and venetoclax, as well as anticipated venetoclax mechanism-based lymphopenia that may potentially increase the risk of opportunistic infections).
- Inability to swallow oral medications
- Any malabsorption condition
- Treatment with strong and moderate CYP3A inhibitors (see Appendix 1) during screening
- Cardiovascular disability status of New York Heart Association (NYHA) Class ≥ 2.
  Class 2 is defined as cardiac disease in which patients are comfortable at rest but ordinary physical activity results in fatigue, palpitations, dyspnea, or anginal pain.
- Chronic respiratory disease that requires continuous oxygen use.
- White blood cell count > 25 × 10^9/L. Note: Hydroxyurea is permitted to meet this criterion.
- AML relapse treatment with any investigational or commercial drug within 14 days before enrolment. Hydroxyurea is allowed until enrolment to control peripheral WBC counts. Toxic effects of previous investigational drug treatment have to recover to Grade <2.
- Substance abuse, medical, psychological, or social conditions that may interfere with the subject's cooperation with the requirements of the trial or evaluation of the study results
- Pregnant or breastfeeding women.
  Breastfeeding has to be discontinued before onset of and during treatment and should be discontinued for at least 3 months after end of treatment.
- History of active or chronic infectious hepatitis unless serology demonstrates clearance of infection
  (Occult or prior hepatitis B virus (HBV) infection (defined as negative hepatitis B surface antigen and positive total hepatitis B core antibody) may be included if HBV DNA is undetectable, provided that they are willing to undergo monthly DNA testing. Patients who have protective titers of hepatitis B surface antibody after vaccination or prior but cured hepatitis B are eligible. Patients positive for hepatitis C virus antibody are eligible provided PCR is negative for HCV RNA.)
- History of clinically significant liver cirrhosis (e.g., Child-Pugh class B and C).
- Live-virus vaccines given within 28 days prior to the initiation of study treatment

4.3 Justification for gender selection

Men and women (postmenopausal or using appropriate contraception) will be enrolled in this study. The proportion of men and women enrolled in this study depends only on the availability of subjects at the study site. Due to the severity of the underlying disease, the treatment of men as well as women is justified. There is no data suggesting increased or decreased activity of the study drug in either gender.

4.4 Withdrawal of subjects from study

4.4.1 Withdrawal

Participation in the trial is voluntary. A subject has the right to withdraw from the study at any time for any reason without any consequences for the further medical treatment. If s/he chooses to withdraw, the investigator will be informed immediately. After withdrawal of the study consent, the subject will be treated according to standard of care. Furthermore, the investigator has the right to terminate the participation of any subject at any time, if s/he deems it in the participant's best interest. The reason and circumstances for study discontinuation will be documented in the participant's eCRF.

Reasons for patient's premature end of study might be:

- At their own request or at the request of their legally acceptable representative
- If, in the investigator's opinion, continuation of study would be harmful to the subject's wellbeing or if the subject is no longer eligible for the study (e.g. development of any intercurrent illness).
- At specific request of the sponsor and in liaison with the investigator (e.g. obvious non-compliance, safety concerns)
- Use of illicit drugs or other substances abuse that may, in the opinion of the investigator, have a reasonable chance of contributing to toxicity or otherwise confound the results
- Subjects who experience a relapse or are diagnosed with resistant disease throughout the trial, unless the investigator (in consultation with the sponsor) deems that continued treatment is appropriate.
- The development of a second malignancy that requires a different treatment
- Interruption of venetoclax >28 days during maintenance therapy

A withdrawn subject is referred to as either "screening failure" or "dropout (= premature end of treatment)" depending on the time point of withdrawal.

A subject who discontinues study treatment prematurely for any reason is defined as a “dropout” if the subject has already been administered at least 1 dose of the study drug. Data of drop-out patients will be analyzed intention-to treat as part of the full analysis set (FAS), see section 8.2.2.1.

A subject who, for any reason (e.g. failure to satisfy the selection criteria), terminates the study prior to the first dose of study drug is regarded as a “screening failure”.

In all cases, the reason for withdrawal must be recorded in the electronic case report form (eCRF) and the subject’s medical record. If a patient has withdrawn his consent, it has also to be documented in the subject’s medical record whether the patient has agreed to further documentation of personal data during follow-up period.

Treatment with the Investigational medicinal product (IMP) venetoclax should be stopped for the following reasons and has to be reported in the appropriate page of the eCRF:

- Severe allergic reactions, such as exfoliate erythroderma, anaphylaxis, or vascular collapse, related to the study drug
- Experience of DLT
- Subjects with a beta human chorionic gonadotropin test consistent with pregnancy. Pregnancy will be reported along the same timelines as SAEs (see section Reporting of SAEs and SARs).
- Intolerable toxicity or recurrent toxicities requiring dose reduction venetoclax below 50 mg or 10 mg

These patients stay in the trial and have to perform all visits and assessments according to visit schedule until V8 – Remission Assessment.

Details for the premature termination of the whole study are provided in section 10.

4.4.2 Replacement

Subjects who experience a DLT will NOT be replaced.

In the dose escalation part of the trial (i.e. phase I), following patients should be replaced to ensure the required number of evaluable subjects per cohort:

- subjects not experiencing a DLT who have taken less than the required study treatment as described in section 3.2.1
- subjects with incomplete DLT evaluation period due to any reason other than a DLT even if they have received the required study treatment (section 3.2.1)

In the expansion part of the trial (i.e. phase II), subjects will not be replaced.

5 Study treatment

5.1 Investigational medicinal product (IMP) venetoclax

5.1.1 Description of the IMP

The study drug provided is venetoclax. The study drug will be supplied as 10mg, 50 mg and 100 mg film coated tablets in blisters or bottles. See current Investigator's Brochure for further characteristics, mechanism of action, preclinical activity, side effects, pharmacokinetics and results of clinical trials.

5.1.2 Packaging and labeling

The pharmaceutical manufacturer AbbVie is responsible for quality control, labelling, batch release, packaging and shipment to the central distribution pharmacy. Venetoclax will be labeled according to the requirements of local law and legislation. Label text will be approved according to the Sponsor's procedures. A complete record of batch numbers and expiry dates of all study treatment will be maintained in the sponsor study file.

The clinical trial sites will be supplied with a venetoclax supply sufficient for one full cycle of V-MAC for one patient, i.e.

- 1 blister of "***8 x 50mg tablets, child resistant blister cards***" (sufficient for days 1-3) and
- 4/6 blisters of "***8 x 100 mg tablets, child resistant blister cards***" (sufficient for days 4-10/14)

For maintenance treatment, the clinical trial sites will be supplied with 3 bottles, each containing "***120 x 100mg tablets, child resistant bottles***", re-supply every 3 months on request of trial site.

If a dose reduction of venetoclax is necessary because of co-administration of strong and/or moderate CYP3A inhibitors during the expansion phase and maintenance, the patient will be supplied with sufficient blisters of "***16 x 10mg tablets, child resistant blister cards***".

Each blister or bottle carries a label as shown in Figure1, depending on the dose it contains.

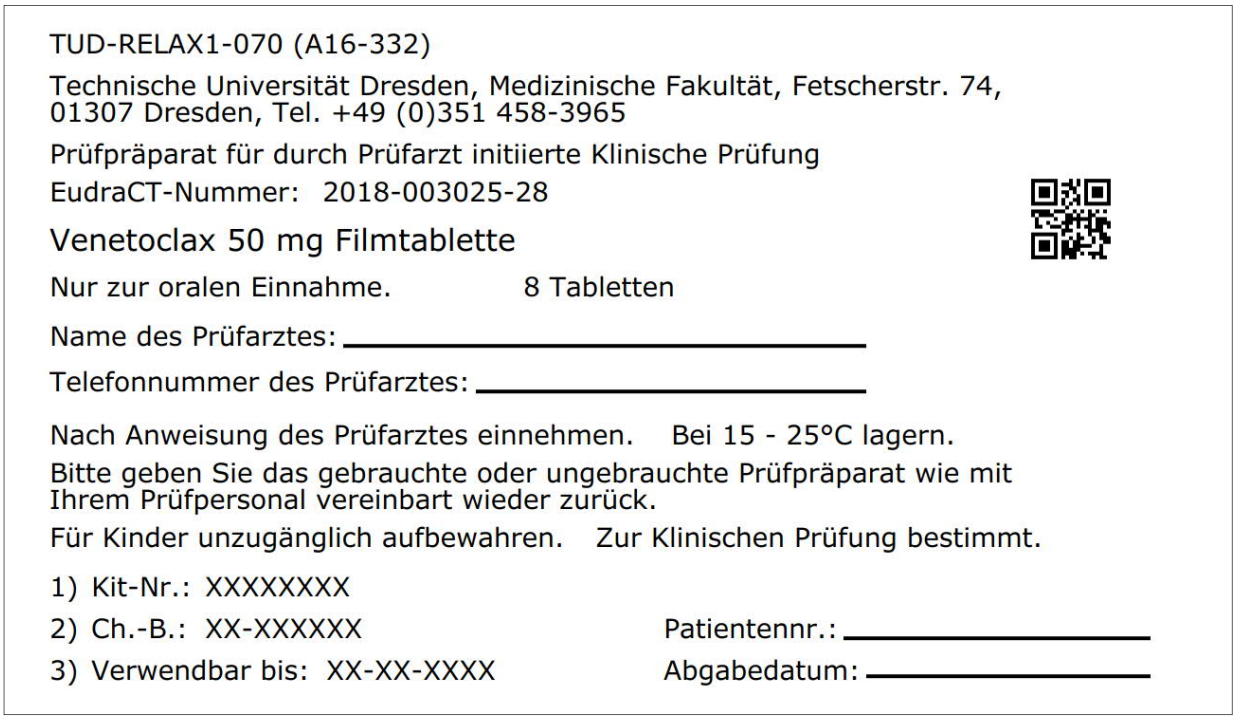
TUD-RELAX1-070 (A16-332)
Technische Universität Dresden, Medizinische Fakultät, Fetscherstr. 74, 01307 Dresden, Tel. +49 (0)351 458-3965
Prüfpräparat für durch Prüfarzt initiierte Klinische Prüfung
EudraCT-Nummer: 2018-003025-28
Venetoclax 50 mg Filmtablette
Nur zur oralen Einnahme. 8 Tabletten
Name des Prüfarztes: _____
Telefonnummer des Prüfarztes: _____
Nach Anweisung des Prüfarztes einnehmen. Bei 15 - 25°C lagern.
Bitte geben Sie das gebrauchte oder ungebrauchte Prüfpräparat wie mit Ihrem Prüfpersonal vereinbart wieder zurück.
Für Kinder unzugänglich aufbewahren. Zur Klinischen Prüfung bestimmt.
1) Kit-Nr.: XXXXXXXX
2) Ch.-B.: XX-XXXXXX Patientennr.: _____
3) Verwendbar bis: XX-XX-XXXX Abgabedatum: _____

Figure 1: sample of packaging label of venetoclax

5.1.3 Provision, storage and dispensing

The central distribution pharmacy will distribute the labeled study medication to the trial sites. The sites will be supplied with venetoclax supply sufficient for one full cycle of V-MAC for one patient after initiation of the study. Sites are responsible for contacting the drug distribution center to order study drug for further treatment of individual patients in the trial. Sites will complete the Drug Request Form available in the ISF. The form will be faxed or emailed to the sponsor's drug distribution center. The faxes/emails are processed during regular business hours, Monday to Friday 08:00 am to 05:00 pm.

Storage and dispensing

Blister packs and bottles should be stored at 15-25°C. After dispensing to the patient, venetoclax may be stored at home at room temperature (below 25°C) for as long as patient's study participation.

The site's investigator is responsible for ensuring the correct storage and sufficient stocks of the IMP at the site.

5.1.4 Drug accountability

The investigator has to ensure that the investigational medicinal product (IMP) is only used according to this protocol.

- The investigator bears the responsibility for the proper storage in an appropriate place to which unauthorised persons have no access and storage conditions are kept.
- The investigator must only dispense the IMP to subjects who have been enrolled in the study. The dispensing of the IMP to subjects outside of this clinical trial is not permitted.
- The investigator, or an individual who is designated by the investigator per delegation log, should explain the correct use of the IMP to each trial subject and check at regular intervals that each subject is following the instructions correctly.

Investigational medicinal product Stock Lists (Drug Accountability)

The investigator or pharmacist or another appropriate individual who is designated by the investigator, should maintain records of the stocks of the IMP, the use by the individual trial subjects, and the return of unused investigational medicinal products to the trial centre or its disposal.

- Each delivery will be documented in writing on the forms provided and the forms will be returned to the Drug Distribution Center stated as an acknowledgement of receipt.
- Complete documentation of the whereabouts of the IMP (incoming/outgoing) on appropriate forms is mandatory. These records should include dates, quantities, batch numbers, expiry dates (if applicable) and the unique code numbers assigned to the IMP and the trial subjects. The investigator should maintain records that document adequately that the subjects received the doses specified by the protocol. These records should be checked against all delivery notes received from AbbVie.
- Any discrepancies between stock and drug account have to be explained.
- The Investigator is responsible for destroying any unused, partially used and empty blisters/bottles of IMP after the clinical monitor has approved the drug accountability logs. This process should be documented appropriately. A confirmation of the destruction will be filed in Investigator’s Site File and also in the TMF.

The patients should document the administration of the study drug in a patient diary which will be provided from the sponsor.

5.2 Cytarabine

Commercially available cytarabine will be given and provided locally.

For information on the formulation, packaging, and handling of cytarabine see the summary of product characteristics (SmPC) for cytarabine as used in standard practice.

Cytarabine will be defined as Investigational medicinal product (IMP) for Phase I/Escalation Phase only, in Phase II/Expansion Phase, it will be given as concomitant medication.

5.3 Mitoxantrone

Mitoxantrone will be given as concomitant medication and supplied locally. For information on the formulation, packaging, and handling of mitoxantrone see the the SmPC for mitoxantrone as used in standard practice.

5.4 Dosing and administration

The treatment plan combines a fixed dose of venetoclax and mitoxantrone with increasing doses of cytarabine (V-MAC). Patients will be admitted to the hospital for at least the first week of study treatment.

5.4.1 Treatment schedule in dose escalation part of the study (phase I)

The treatment schedule will be as follows:

Table 1: Treatment schedule in the dose escalation part

Dose level	Venetoclax target dose QD	Cytarabine dose	Mitoxantrone dose QD
-1	50-400 mg PO, days 1-10	100 mg/m^2 IV CI, days 4-10	10 mg/m^2 IV, days 6-8
1	50-400 mg PO, days 1-14	200 mg/m^2 IV CI, days 4-10	10 mg/m^2 IV days 6-8
2	50-400 mg PO, days 1-14	500 mg/m^2 IV BID, days 4-6	10 mg/m^2 IV days 6-8
3	50-400 mg PO, days 1-14	1000 mg/m^2 IV BID, days 4-6	10 mg/m^2 IV days 6-8

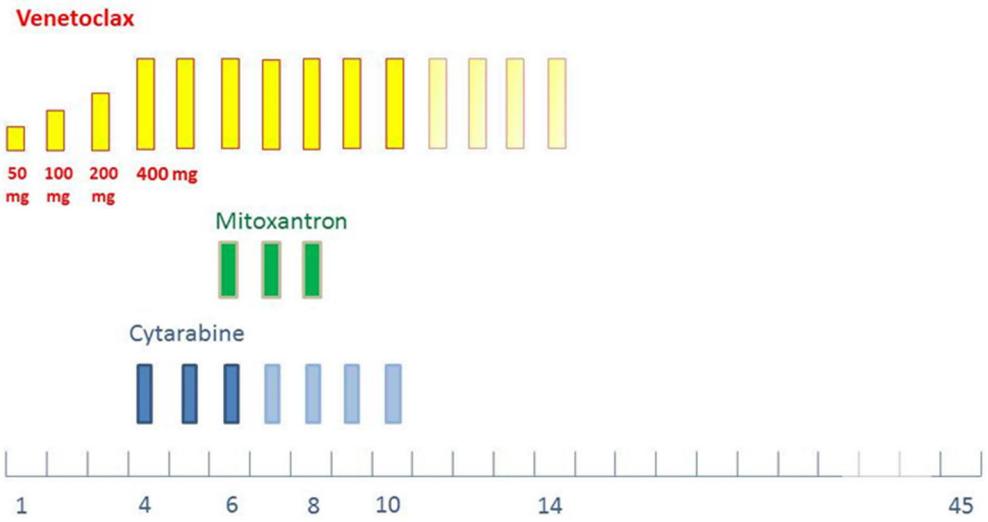

Figure 2: Treatment schedule

Venetoclax

Day 1 of venetoclax will define the study treatment start. To mitigate the potential risk of TLS, venetoclax administration will start with a pre-specified dose ramp-up to reach the target dose level of 400 mg at day 4 (refer to Table 2).

Table 2: Venetoclax ramp up

Day	Venetoclax dose
1	50 mg
2	100 mg
3	200 mg
4 – 14 (10)*	400 mg

* Venetoclax will be administered until day 14, only at dose level -1, venetoclax will be given until day 10.

Each dose of venetoclax should be taken with approximately 240 ml of water within 30 minutes after the completion of a meal, preferably breakfast. The dose should be administered at the same time each day. On days with planned cytarabine administration, venetoclax must be given prior to cytarabine and mitoxantrone. If a patient develops any lab changes suggestive of TLS within the first 24 hours after either first dose or during ramp up, venetoclax should be withheld until normalization of lab changes. Venetoclax will be administered in Phase I and II no longer than 10/14 days except during maintenance therapy.

Cytarabine

Day 4-6 (10) 100 - 1000 mg/sqm (according to dose level)

Cytarabine will be given at increasing doses and should be administered after venetoclax intake. In the dose escalation part of the trial, cytarabine will be given at increasing doses as outlined in Table 1 starting from day 4. Cytarabine will be administered as continuous infusion in dose levels -1 and 1, by IV infusion over 2 hours twice daily in dose levels 2 and 3.

Mitoxantrone

Day 6-8 10 mg/m^2 IV over 1 hour

Mitoxantrone will be administered by IV infusion over 1 hour on day 6, 7 and 8 parallel to cytarabine in cohort -1 and 1, after cytarabine infusion in cohorts 2 and 3 (see Figure 2).

5.4.2 Treatment schedule in the expansion part of the study (phase II)

Venetoclax

Venetoclax will start on day 1 and continue until day 14 (10) with a ramp up period as outlined in Table 2 in section 5.4.1.

In case of co-administration with posaconazole and other strong and moderate CYP3A inhibitors, dose modifications of venetoclax are required as described in section 5.5.

Cytarabine

Cytarabine will be given at the MTD or highest tolerable dose determined in the dose escalation part. Administration conditions will be identical to the dose escalation part of the trial (see 5.4.1).

Mitoxantrone

Day 6-8 10 mg/m^2 IV over 1 hour

Mitoxantrone will be administered by IV infusion over 1 hour on day 6, 7 and 8.

5.4.3 Treatment schedule in the maintenance therapy

Patients who achieve a CR/CRp with ANC recovery (ANC ≥ 1000/µL/≥1 x 10^9/L) may receive venetoclax maintenance treatment up to twelve 28-day cycles if they have no other post- remission treatment options (e.g. auto/allo-TX, chemo-consolidation therapy etc.).

Venetoclax will be given at dose 400 mg QD per os as continuous treatment starting within 14 days after the End of Treatment assessment of previous treatment phase up to 12 cycles. Duration of one cycle is 28 days.

If co-administration with strong and moderate CYP3A inhibitors daily dose will be reduced according to section 5.5.

5.4.4 Dose modifications during V-MAC in expansion and maintenance treatment

The following dose reduction rules shall be applied in case toxicities occur.
In case of either one of these toxicities, venetoclax shall be stopped and re-started according to the recommended dose modifications in Table 3 and Table 4. If co-administration of strong or moderate CYP3A inhibitors, venetoclax dose modification has to be done according to Table 5 and Table 6.

In maintenance therapy, dose reductions according to Table 3, Table 4, Table 5, Table 6 are necessary if either one of the below occurs in patients with CRp:

- ANC falls <1000/µL or
- PLT count falls <50,000/µL or
- Toxicities grade ≥ 3

Table 3: Recommended Dose Modifications for Toxicities during V-MAC in expansion and maintenance

Event	**Occurrence**	**Action**
Non-Hematologic Toxicities		
Grade 3 or 4 non-hematologic toxicities	1st occurrence	Interrupt venetoclax. Once the toxicity has resolved to Grade 1 or baseline level, venetoclax therapy may be resumed at the same dose. No dose modification is required.
	2nd and subsequent occurrences	Interrupt venetoclax. Follow dose reduction guidelines in Table 4 when resuming treatment with venetoclax after event has resolved to grade 1.

Hematologic Toxicities (only during maintenance)		
Grade 3 or 4 neutropenia with infection or fever; or Grade 4 hematologic toxicities (except lymphopenia)	1st occurrence	Interrupt venetoclax. To reduce the infection risks associated with neutropenia, granulocyte-colony stimulating factor (G-CSF) may be administered with venetoclax if clinically indicated. Once the toxicity has resolved to Grade 1 or level on day 1 of maintenance, venetoclax therapy may be resumed at the same dose.
	2nd and subsequent occurrences	Interrupt venetoclax. Consider using G-CSF as clinically indicated. Follow dose reduction guidelines in Table 4 when resuming treatment with venetoclax after event has resolved to Grade 1 or on day 1 of maintenance.

Table 4: Dose Modification for Toxicity during Venetoclax Treatment

Dose at interruption, mg	**Restart dose, mg**
400	300
300	200
200	100
100	50
50	Stop study drug permanently

Table 5: Dose Modification for Toxicity during Venetoclax Treatment in case of **Co-medication with strong CYP3A inhibitors**

Dose at interruption, mg	**Restart dose, mg**
100	50
50	20
20	10
10	Stop study drug permanently

Table 6: Dose Modification for Toxicity during Venetoclax Treatment in case of **Co-medication with moderate CYP3A inhibitors**

Dose at interruption, mg	Restart dose, mg
200	100
100	50
50	20
20	10
10	Stop study drug permanently

5.4.5 Prophylaxis and Management of Tumor Lysis Syndrome (TLS)

There is a potential for TLS in all subjects with AML, especially in those with elevated pretreatment LDH levels, elevated leukocyte count, renal dysfunction and dehydration. To mitigate the risk for TLS (Coiffier et al., J Clin Oncol 2008), subjects will receive tumor lysis prophylaxis, including hydration (e.g., oral, intravenous) and treatment with an agent to reduce the uric acid level (e.g., allopurinol, rasburicase) (Coiffier et al., J Clin Oncol 2008, Cairo et al., Br J Haematol 2004) prior to and during the ramp up period during escalation part. For subjects who had dose delay or interruptions, TLS prophylactic measures may need to be implemented based on the disease status prior to resuming treatment.

TLS Prophylaxis and Management in Escalation part:

TLS prophylaxis must be initiated in all such subjects prior to the first venetoclax dose or first new escalated dose as described below.

- Hospitalization (required) starting the night before the first dose of venetoclax and for a minimum of 24 hours after reaching the target dose of 400 mg venetoclax.
- An oral agent to reduce the uric acid level (e.g., allopurinol) to be initiated at least 72 hours prior to dosing. Treatment may need to be continued for up to 28 days based on the ongoing risk of TLS development. Subjects allergic to or otherwise unable to receive allopurinol must use another uric acid reducer starting at least 72 hours prior to dosing or rasburicase on the day of treatment (prior to venetoclax dosing).
- Oral hydration (approximately 1.5 to 2 liters as tolerated) per day starting from at least 24 hours prior to first dose or any dose escalation during ramp up.
- Intravenous hydration must be started upon admission or the night prior to beginning treatment with a target of approximately 2 L per day or as clinically appropriate and continued during

hospitalization. Urine output must be monitored. Diuretics may be used per investigator discretion.

- TLS chemistry tests on the first day of venetoclax dosing and each day of a new dose at pre-dose (within 4 hours prior to dosing), 6-8 hours and 24 (± 2) hours post-dose. Additional laboratory assessments may be performed, per investigator discretion, post-dose during ramp up and up to 48 hours after reaching the target dose if clinically indicated.
- TLS chemistry test results (calcium, inorganic phosphorus, potassium, uric acid, and creatinine) must be reviewed by the investigator in real time and prior to the subject's next dose of venetoclax to ensure appropriate subject management.
- If any clinically significant laboratory changes are observed within the first 24 hours after initiation of dosing or after a dose escalation during ramp up, see Appendix 2 (Recommendations for Initial Management of Electrolyte Abnormalities and Prevention of Tumor Lysis Syndrome [TLS]) for management guidelines. Refer to table in Appendix 3 for definitions of laboratory and clinical TLS.
- If a subject meets criteria for clinically significant laboratory TLS, no additional venetoclax dose should be administered until resolution.
    - Prophylactic reductions of potassium, inorganic phosphorus and/or uric acid above normal range prior to beginning dosing are recommended.

For continued dosing of venetoclax, monitor for evidence of TLS during treatment, and manage abnormalities in serum creatinine, uric acid and electrolytes promptly. For subjects at higher risk (i.e., circulating blasts), more intensive measures should be considered. Refer to Appendix 2 for a special sample handling procedure that must be followed to avoid ex vivo uric acid degradation in the presence of rasburicase.

If the chemical changes and laboratory TLS have not resolved within 24 hours, the continuation of venetoclax has to be discussed between the investigator and the Coordinating Investigator.

TLS Prophylaxis and Management in the Expansion part

Safety data from the 94 subjects treated in 3 AML studies demonstrated no events of TLS; 32 subjects in monotherapy study (Study M14-212) and 22 subjects in a combination study (Study M14-387) using low dose cytarabine. Based on this data, simplification of TLS prophylaxis and management will be followed for subjects enrolled into the expansion stage.

All subjects enrolled into the expansion part will need TLS prophylaxis and monitoring. The minimum requirements for TLS prophylaxis and management for subjects enrolled into the

expansion part are shown below. All other prophylaxis and monitoring procedures for TLS will be done as per institutional/regional standards:

- All subjects will be hospitalized on or before Day 1 of study treatment (V-MAC) prior to administration of the first dose of study venetoclax and remain in the hospital at least for 24 hours after reaching the target dose of 400 mg venetoclax.
- Administration of uric acid reducing agent, adequate oral and intravenous hydration while monitoring the fluid status of the subject prior to and during the ramp up of venetoclax will be based on regional standards or institutional guidelines.
- TLS chemistry tests are to be drawn (calcium, inorganic phosphorus, potassium, uric acid, and creatinine) on the first day of venetoclax dosing and on each day of a new dose at pre-dose (within 4 hours prior to dosing), 6-8 and 24 (± 2) hours post dose. Additional laboratory assessments may be performed, per investigator discretion, post-dose during ramp up and up to 48 hours after reaching target dose if clinically indicated.
- Abnormal chemistry test results should be corrected promptly.
- If a subject meets criteria for clinically significant laboratory or clinical TLS, no additional venetoclax dose should be administered until resolution.
    - Prophylactic reductions of potassium, inorganic phosphorus and/or uric acid above normal range are recommended prior to beginning study treatment and continue based on the ongoing risk of TLS.

For continued dosing of venetoclax, monitor for evidence of TLS during treatment, and manage abnormalities in serum creatinine, uric acid and electrolytes promptly. For subjects at higher risk (i.e., circulating blasts), more intensive measures should be considered.

Drug interruption for up to 72 hours following transient (i.e., lasting < 48 hours) chemical changes and laboratory TLS may be allowed and will not require a dose reduction. If the TLS has not resolved within 72 hours, then a dose reduction should be considered.

5.4.6 Dose modifications in obese patients

There is no clearly documented adverse impact of treatment of obese patients when dosing is performed according to actual body weight. Therefore, all dosing is to be determined solely by the patient's BSA as calculated from actual weight. This will eliminate the risk of calculation error and the possible introduction of variability in dose administration. Failure to use actual body weight in the calculation of drug dosages will be considered a major protocol deviation. Physicians who are

uncomfortable with administering chemotherapy dose based on actual body weight should not enroll obese patients.

5.5 Concomitant therapy

All concomitant medications (including start / stop dates, total daily dose, and indication) must be recorded in the subject's source documentation as well as in the appropriate pages of the eCRF. This includes also carrier solution of chemotherapy, hydration, antiemetic therapy, and contrast media, which might be administered during assessments.

For subjects at risk for developing a tumor lysis syndrome (TLS), TLS prophylaxis according to standard local practice with (aggressive) hydration, allopurinol, and/or rasburicase is recommended.

Not permitted:

General guidelines regarding excluded, cautionary and allowed medications are summarized in Table 7.

Table 7: Excluded and Cautionary Medications and Dietary Restrictions
(See Appendix 1 for Examples of the Medications)

Excluded Foods within 3 days prior to enrolment, During Ramp-Up and Throughout Study:
• Grapefruit and grapefruit products • Seville Oranges (including marmalade containing Seville oranges) • Starfruit
Excluded Medications During Escalation phase:
• Strong and moderate CYP3A inhibitors • Strong and moderate CYP3A inducers
Cautionary during Expansion phase and Maintenance
• Warfarin* • CYP3A inducers • Strong, moderate and weak CYP3A inhibitors. In case of co-administration of venetoclax and strong and moderate CYP3A inhibitor, a dose reduction of venetoclax is necessary (see Table 8 and 9) • P-gp substrates • BCRP substrates • OATP1B1/1B3 substrates • P-gp inhibitors • BCRP inhibitors

* Closely monitor the international normalized ratio (INR).

A sample list of excluded medications and cautionary medications that fall into the categories can be found in Appendix 1. It is not possible to produce a 100% exhaustive list of medications that fall into these categories, so if in question, please refer to the appropriate product label.

Note: In patients with suspected fungal infection during venetoclax days, treatment with caspofungine or liposomal amphotericine B is recommended.

Expansion Phase:

For the phase 2 part of the protocol, the below dose modification according to the following Table 8 is recommended in case of co-administration with strong / moderate CYP3A inhibitors.

Table 8: Venetoclax in case of strong and moderate CYP3A inhibitors

Inhibitors	Initiation and Ramp-Up	Venetoclax Daily Dose (after Ramp-Up)
Strong CYP3A inhibitor	Day 1 – 10 mg Day 2 – 20 mg Day 3 – 50 mg Day 4 – 100 mg or less	100 mg or less
Moderate CYP3A inhibitor (reduce the Venetoclax dose by at least 50%)	Day 1 – 20 mg Day 2 – 50 mg Day 3 – 100 mg Day 4 – 200 mg	200 mg

5.6 Post-study therapy

The leukemia treatment after the end of the study (defined for different phases see section 3.7) will be left to the discretion of the investigator and / or the primary physician and the subjects will be treated according to standard of care (e.g. consolidation therapy or allogeneic SCT).

5.7 Pregnancies

The investigator must report to the sponsor any pregnancy occurring in a study subject, or in his partner, during the subject's participation in this study until 28 days after last administration of the study drug. The report should be submitted within the same timelines as an SAE, although a pregnancy per se is not considered an SAE. For a study subject, the outcome of the pregnancy should be followed-up carefully, and any abnormal outcome of the mother or the child should be reported. For the pregnancy of a study subject's partner, all efforts should be made to obtain similar information on course and outcome, subject to the partner's consent. For all reports, the forms provided are to be used.

6 Study procedures

6.1 Study flowcharts and visit schedules

Table 9: Visit schedule Escalation and Expansion part

	V0 – Screening	Enrolment	V1	V2	V3	V4	V5	V6	V7	V8 – Remission Assessment[i]	Premature EOS	Post-study Follow-up[k]
Study – Day	**-14 to -1**	**1 (predose)**	**1**	**2-6 daily**	**7**	**9**	**11**	**18**	**22**	**28-45 (all patients)**	**7-14 days after last dose for safety**	**Every 3 months**
Informed Consent	x											
Check Inclusion/Exclusion Criteria		x										
Demographics, Medical/Treatment History	x											
Physical exam, ECOG, Vital Signs[l]	x		x		x			x		x	x	
body weight and height[m]	x		x							x	x	
Full Blood Count[a]	x		x	x	x	x	x	x	x	x	x	
Chemistry and Coagulation[b,c]	x		x		x	x	x	x	x	x	x	
TLS chemistry tests[d]			x	x								
Bone marrow assessment[e]	x							x[f]		x	x if progress	
Material shipment for central Review[g]	x							x		x	x if progress	
HIV-Test	x											
Virus diagnostics[n]	x											
β-HCG pregnancy test[h]	x											
ancillary research material[o]	x									x	x if progress	
Adverse Events			Continuously starting from day 1 until 28 days after last dose of venetoclax									
Concomitant Medication			Continuously starting from day 1 until 28 days after last dose of venetoclax									
Disease status & Survival												x

[a] Blood count (hemoglobin, hematocrit, white blood cells, platelets) incl. differential - for screening and remission assessment microscopic differential is required for peripheral blasts. In addition, blood count should be done 3x/week until hematologic recovery and as often as necessary in the opinion of the treating physician

[b] Determination of: sodium, potassium, calcium, creatinine, urea, ALAT (GPT), ASAT (GOT), LDH, alkaline phosphatase, bilirubin, total protein, albumin, uric acid, INR, aPTT, fibrinogen.

[c] All subjects must receive tumor lysis prophylaxis prior to and during treatment, including chemistries at 24, 48 and 72 hours after administration of the designated cohort dose. For details on tumor lysis prophylaxis and management, refer to section 5.4.5 Management of Tumor Lysis Syndrome (TLS) and Appendix 2 – Recommendations for Initial Management of Electrolyte Imbalances and Prevention of Tumor Lysis Syndrome (TLS) for further information. Subjects in the expansion stage only require TLS prophylaxis and management 24, 48, and 72 hours after administration of the designated cohort dose, if clinically necessary.

[d] TLS chemistry tests (calcium, inorganic phosphorus, potassium, uric acid, creatinine) on each day of new dose of venetoclax at pre-dose, 6-8 hours and 24 (± 2) hours post-dose (see section 5.4.5)

[e] local bone marrow assessment: cytomorphology including cytochemistry, immunophenotyping, cytogenetics, molecular diagnostics

[f] day 18: only BM-assessment for cytomorphology incl. cytochemistry

[g] samples for central diagnostics (Hema Lab Dresden): 5 bone marrow and 2 peripheral blood smear slides for cytomorphology and 10ml heparinized bone marrow for immunophenotyping

[h] Women of childbearing potential must have a negative serum pregnancy test performed **within 7 days** before first dose of venetoclax

[i] Remission Assessment-visit on day 28-45 is required for all patients regardless a subject has prematurely terminated the study treatment for any reason or not (except for death or withdrawn ICF).

[k] All patients will be followed-up after last study visit for survival, remission status, further AML therapies (regimes and start dates) and second primary malignancies for minimum 24 months, either by phone calls or visits.

[l] vital signs: blood pressure, heart frequency and temperature

[m] height only at Screening

[n] Virus diagnostics (according to clinical standards): HBs-antigen, HBs-antibody (if positive HBs-antibody – HBV DNA required), anti-HBc, anti-HCV IgG (if positive anti-HCV IgG – HCV RNA required)

[o] ancillary research: collection of 9 ml peripheral blood (pB) in EDTA-tube and 5 ml bone marrow (BM) in EDTA-tube + 3 ml heparinized BM at screening, remission assessment and in case of progress (see section 6.5)

Table 10: Visit schedule maintenance therapy

	Maintenance pre-visit (only done, if V8 of previous treatment phase >14 days)	**Eligibility**	**Cycle 1**		**End of Cycle 2, 4, 6, 9**	**End of Cycle 12 = EOS or at Premature EOS[f]**	**Post-study Follow - up[g]**
Cycle day		**day -7 to -1**	**d1**	**d15**	**d28 (+/-5days)**	**d28 (+/-5days)**	**every 3 months**
Physical exam, ECOG	x		x	x	x	x	
Vital Signs, body weight[h]	x		x	x	x	x	
Full Blood Count[a]	x		x	x	x	x	
Chemistry and Coagulation[b]	x		x	x	x	x	
β-HCG pregnancy test		x					
Bone marrow assessment[c,d]	x				x	x	
Material for central Review[e]	x				x	x	
ancillary research material[i]					x	x	
Concomitant Medication	x		continuous till day 28 after last dose of IMP				
Adverse Events	x		continuous till day 28 after last dose of IMP				
Disease status & Survival							x

[a] Blood count (hemoglobin, hematocrit, white blood cells, platelets) incl. microscopic differential except C1D15. In addition, these evaluations should be done as often as necessary in the opinion of the treating physician

[b] Determination of: sodium, potassium, calcium, creatinine, urea, ALAT (GPT), ASAT (GOT), LDH, alkaline phosphatase, bilirubin, total protein, albumin, uric acid, INR, aPTT, fibrinogen.

[c] local bone marrow assessment: cytomorphology including cytochemistry, immunophenotyping, cytogenetics, molecular diagnostics

[d] Bone marrow assessment and peripheral complete blood count proving a CR/CRp (not CRi) is a prerequisite for the start of maintenance treatment. If CR/CRp is proven at the V8 and maintenance starts within 2 weeks after bone marrow assessment, no additional bone marrow is necessary.

[e] samples for central diagnostics (Hema Lab Dresden): 5 bone marrow and 2 peripheral blood smear slides for cytomorphology and 10 ml heparinized bone marrow for immunophenotyping

[f] If a subject discontinues the study for any reason (except for death or loss to follow-up), a premature end-of-study evaluation will be performed 7 to 14 days after the last dose of study drug.

[g] All patients will be followed-up after last study visit for survival, remission status, further AML therapies (regimes and start dates) and second primary malignancies for minimum 24 months, either by phone calls or visits

[h] vital signs: blood pressure, heart frequency and temperature

[i] ancillary research: collection of 9 ml peripheral blood (pB) in EDTA-tube at end of cycle 2, 6 and 12; in case of relapse 9 ml pB and 5 ml bone marrow (BM) in EDTA-tube + 3 ml heparinized BM (see section 6.5)

6.2 Study visits and diagnostic procedures

The table above lists only procedures which are specifically required for the evaluation of study aims and endpoints. Additional diagnostic and therapeutic procedures may be necessary according to clinical standard as defined in evidence-based AML treatment guidelines [NCCN guideline, Onkopedia-Leitlinie, Döhner et al 2010] and according to the investigator's clinical experience and expertise.

6.3 Assessment of safety

Regular safety assessment will ensure that patients have no or minimal study related risk. Safety evaluations will include results of physical examinations (including neurological disorders), vital signs (blood pressure, heart frequency, temperature), AEs, concomitant medications, and abnormal laboratory tests.

The following parameters will be used for safety assessment:

- Rate of early deaths (within 14 and 30 days) and induction deaths (until day 60 or until the beginning of next treatment – whichever occurs first)
- Incidence of serious infectious complications (Grades 3-5 CTCAE V5.0)

6.4 Assessment of efficacy

Response and progression of leukemia subjects will be evaluated in this study based on local results of bone marrow aspirates / biopsies / peripheral whole blood. Response assessment in this study will be based on the revised recommendations of the International Working Group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic studies in AML (Cheson et al, 2003, Döhner et al, Blood 2017).

A central review will be performed for cytomorphological examinations and immunophenotyping for MRD assessment in the Hematology Lab in Dresden and is used for quality control.

6.4.1 Bone marrow assessments

Bone marrow assessments should be done preferentially as aspirations. Bone marrow biopsies are only necessary in case of dry puncture and the inability to aspirate bone marrow ("punctio sicca").

Bone marrow assessment includes:

- Cytomorphological examination including cytochemistry
- Immunophenotyping
- Cytogenetics (incl. FISH)
- Molecular genetic analyzes for the presence of FLT3-ITD mutation, genomic ratio of the presence of FLT3-ITD vs. FLT3-WT, for nucleophosmin mutations (NPM1) including

NPM1 MRD, for the presence of BCR-ABL1, PML-RARα, RUNX1-RUNX1T1 and CBFß-MYH11, double-CEBPA , ASXL1, RUNX1, TP53 mutations.

The quality of all participating laboratories will be assured by checking the installed quality management systems and checking the availability of certificates for regular participation on External Quality Assessments (Ringversuche).

Central Review

The cytomorphologic examinations and immunophenotyping for MRD analysis (FACS) also will be done centrally in the Hematology Laboratory in Dresden.

The required material for the analyses is:

- ***Cytomorphology:*** at minimum 2 peripheral blood and 2-6 bone marrow smears (unstained)
- ***FACS:*** 10ml heparinized bone marrow

Please ship the samples preferably from Monday – Thursday; shipment on Friday should be avoided.

Shipment Address:

Hämatologisches Labor Haus 65
Medizinische Klinik und Poliklinik I
Universitätsklinikum Carl Gustav Carus
Fetscherstr. 74, 01307 Dresden

If material shipment necessary on Friday, samples should be sent to following address:

Station MK1-S1
Medizinische Klinik und Poliklinik I
Universitätsklinikum Carl Gustav Carus
Fetscherstr. 74, 01307 Dresden

For information on **logistics**, contact the SAL Study Office by phone under +49 (0)351 458 3965 or per Email under annett.haake@ukdd.de .

For information to **cytomorphology** and **flow-cytometry**, contact the morphology review under +49 (0)351 458 19796 or per Email under sal@uniklinikum-dresden.de

6.4.2 Response criteria

6.4.2.1 Early Response Assessment on day 18 – 21 of V-MAC

For early response assessment, only cytomorphology including cytochemistry is required.

Good response:

Reduction in bone marrow blast count to below 5% (aspirate with marrow spicules)

Moderate response:

Reduction in bone marrow blast count, blast count ≥5% (aspirate with marrow spicules)

Refractory disease:

Increase in bone marrow blast count compared to baseline or No change in bone marrow cellularity with unchanged blast count

6.4.2.2 Remission assessment

Remission assessment will follow standard criteria according to Döhner et al., 2017 and will be done 28-45 days after start of V-MAC (depending of blood count recovery), but has to be done latest on day 45 irrespective of the blood count regeneration.

If regeneration blasts are suspected, bone marrow aspiration should be repeated, but not later than day 45 from start of V-MAC. Only the final remission assessment will be documented in the eCRF.

To define the real number of platelets and neutrophils, a period of at least 2 days without transfusion or treatment with G-CSF is necessary to determine a CR or CRi.

Morphologic complete remission (CR):

- Platelet count >100,000/µl
- Granulocyte count of >1,000/µl
- Bone marrow (aspirate with marrow spicules): < 5% blasts,
- Absence of circulating blasts and blasts with Auer rods
- Absence of extramedullary leukemia
- Transfusion independent stable hemoglobin value

Complete remission with incomplete recovery (CRi):

All CR criteria are fulfilled except for residual neutropenia (granulocyte count ≤1000/µl) and/or thrombocytopenia (platelet count ≤100.000/µl).

Complete remission with incomplete platelet recovery (CRp):

All CR criteria are fulfilled except for residual thrombocytopenia (platelet count ≤100.000/µl).

Treatment Failure:

Resistant Disease:

Failure to achieve CR or CRi only includes patients surviving ≥7 days following completion of initial treatment, with evidence of persistent leukemia by blood and/or bone marrow examination

Morphologic relapse:

Reappearance of blasts in PB or bone marrow blasts ≥5% after achievement of complete remission or occurrence of extramedullary disease

Molecular relapse

If MRD can be assessed in the individual patient by multicolour flow cytometry and/or PCR methodology, the patient achieves MRD negative status in CR, the reoccurrence of the previously identified MRD marker in a patient with CR defines a molecular relapse.

Death in aplasia:

Deaths occurring ≥7 days following completion of initial treatment while cytopenic; with an aplastic or hypoplastic bone marrow obtained within 7 days of death, without evidence of persistent leukemia

Death from indeterminate cause:

Deaths occurring before completion of therapy, or <7 days following its completion; or deaths occurring ≥7 days following completion of initial therapy with no blasts in the blood, but no bone marrow examination available

6.5 Ancillary research

Biomarkers for response to venetoclax have not yet been established for patients with AML. Response may depend on the activation of specific intracellular signaling pathways. Identification of biomarkers might help to better interpret results from clinical trials, improve the design of future trials and to identify patient groups who benefit most from this drug. Preliminary data indicate that *FLT3*-Internal Tandem Duplications and Tyrosine-Kinase Domain mutations lead to poor with response to venetoclax[1]. Further, mutations in *TP53*, *WT1*, *PDGFRB*, *ASXL1* and *EZH2* may predict response to venetoclax (Kontro, Leukemia 2017, 31(2):301-309). Notably, a striking selective overexpression of specific HOXA and HOXB gene transcripts were detected in highly BCL-2 inhibitor sensitive AML samples. This is of specific interest since repression of *EZH2* leads to upregulation of *HOX* genes and represents a major mechanism of chemotherapy resistance in AML (Gollner, Nature Medicine, 2017;23(1):553-557). Moreover, venetoclax maintenance therapy may impact on pre-malignant clonal hematopoiesis especially when the founding clones are defined by mutations in chromatin modifiers (Shlush et al., Nature 2014 and Jeziskova et al., Int J Hematol 2015). Therefore, we will set out to study changes in the clonal composition of hematopoiesis in patients who achieved a remission.

We will apply targeted sequencing of genes implicated in AML pathogenesis, chemotherapy resistance and clonal hematopoiesis and perform RNA expression profiling for selected genes. Peripheral blood/bone marrow samples will be collected at screening, in remission and at end of cycle 2, 6 and 12 during maintenance therapy and at relapse/progression of AML according to the following schedule (Table 11):

Table 11: ancillary research – time points of collection

Timepoint / required material	Screening	CR/ CRi	during maintenance therapy			Relapse/ Progression
			End of cycle 2	End of cycle 6	End of cycle 12 = EOT	
Targeted DNA-sequencing and RNA-expression profiling of purified bone marrow (BM) blasts - 5 mL BM in EDTA Tube - 3 mL heparinized BM	x	x				x
Targeted DNA-sequencing of peripheral blood (pB) - 9 mL pB in EDTA Tube	x	x	x	x	x	x

Please ship the samples preferably from Monday – Thursday; shipment on Friday should be avoided.

Shipment Address:

Arbeitsgruppe Immunologie der MK1, Haus 66
Dr. Falk Heidenreich / Dr. Dana Vu Van
Medizinische Klinik und Poliklinik I
Universitätsklinikum Carl Gustav Carus
Fetscherstr. 74, 01307 Dresden

Goals of the ancillary research project are.

1) To determine biomarkers for response and mechanisms of resistance to the combination of venetoclax plus cytarabine based chemotherapy

2) To display the course of minimal residual disease

3) To test if changes in the clonal composition of hematopoiesis of patients occur during maintenance therapy

6.6 Subject identification and registration

Each subject who signs a consent form for the study and starts any screening procedure will be assigned a unique 6-digit number during the screening period. The first 3 digits are site specific; the remaining 3 digits are subject specific and allocated in a sequential order of subjects starting

the screening period. This screening number will be entered into the eCRF regardless of whether the subject is actually treated with study drug.

On day 1, after completion of screening procedures and prior to first dose of venetoclax, the inclusion and exclusion criteria must be checked. If eligibility is confirmed, the patient will be enrolled in the trial and treatment can be started.

If eligibility cannot be confirmed, the respective patient will be defined as screening failure.

The full patient ID number for trial is composed like this:

RELAX – CCC – PP – E1/2

RELAX	prefix labeling patient number as belonging to the RELAX trial
CCC	three digits for local trial center (Kliniknummer)
PP	two digits for patient screening number
E1/2	indicating the enrolment in phase 1 / phase 2

7 Safety reporting

7.1 Definition of adverse events and adverse reactions

7.1.1 Adverse Event (AE)

An **adverse event** is any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.
An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product as part of a clinical study, whether or not related to the medicinal product. Adverse events are to be recorded regardless of their relationship to the study intervention. With respect to intensity, AEs are classified according to the CTCAE classification V5.0.

Protocol-specific clarifications to this definition:

- Abnormal laboratory values should be evaluated by investigator for clinical significance and do not have to be documented as an AE if they are “not clinically significant”. They are to be documented in the database on the laboratory values eCRF form only.
- In order to monitor the safety of the trial participants throughout the trial, AEs need to be documented from first dose of venetoclax until 28 days after the last dose of venetoclax administered in the context of this trial.

- A pathological finding, improved or unchanged in comparison to baseline, does not constitute an AE.
- Symptoms of the disease under study should not be classified as AEs as long as they are within the normal day-to-day fluctuation. Worsening of the underlying disease (relapse or disease progression) or of other preexisting conditions will be recorded as an AE.

Adverse events are classified as either serious or non-serious:

7.1.2 Serious Adverse Event (SAE)

A **Serious Adverse Event** is any untoward medical occurrence that at any dose:

- results in death,
- is life threatening,
- results in persistent or significant disability/incapacity,
- requires inpatient hospitalization or prolongation of existing hospitalization,
- is a congenital anomaly or birth defect,
- is another medically important event (that may require medical treatment to avoid one of the above-mentioned conditions).

Comments:

- Life-threatening in the definition of a SAE or SAR refers to an event in which the subject was at immediate risk of death at the time of event; it does not refer to an event which hypothetically might have caused death if it were more severe.
- Hospitalization means overnight admission.
- Hospitalization without underlying AE does not qualify as SAE. Examples are:
    - Hospitalization for protocol procedures e.g. chemotherapy.
    - Elective hospitalization for a pre-existing condition (i.e. a condition other than the indication for the chemotherapy) that has not worsened.
    - Hospitalization which was already planned at the beginning of the trial. Hospitalization must have been reported at screening visit in the source data and have been performed as planned.
    - Admission to a rehabilitation center or hospice.
    - Hospitalization for administrative or social reasons (e.g. due to anxiety but otherwise treatable on an outpatient basis).
- Medical and scientific judgement should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious.

7.1.3 Adverse Drug Reaction (ADR)

All noxious and unintended responses to a medicinal product related to any dose should be considered ADRs.

The phrase "responses to a medicinal product" means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

7.1.4 Unexpected Adverse Reaction (UAR)

Unexpected Adverse Reaction (UAR) is an adverse reaction which does not meet any criteria for adverse reactions listed as known or possible side effects, neither in type nor in severity or outcome. The reference document for the assessment of expectedness is the current version of the Investigator's Brochure.

7.1.5 Serious Adverse Reaction (SAR)

Serious Adverse Reaction (SAR) is any harmful and unintended reaction possibly related to the study medication regardless of its dosage and which meets at least one of the SAE criteria mentioned above.

7.1.6 Suspected Unexpected Serious Adverse Reaction (SUSAR)

An adverse reaction, for which the nature, severity or outcome is not consistent with the applicable product information, which is serious according to definition in 7.1.2 and where the investigational product is suspected to be a contributor to the event.

7.2 Documentation of AEs and SAEs

AEs observed, mentioned upon open questioning by a member of the investigator team, or spontaneously reported by the subject, will be documented in the patient chart. AEs need also to be documented event based in the eCRF.

The investigator has to record all AEs occurring in the period between day 1 of study treatment (= enrolment) until 28 days after the end of venetoclax treatment on the respective eCRF pages. Patients will be followed-up for disease status and survival for minimum 24 months.

"Death" should not be reported as an SAE, since "death" is the outcome of the underlying SAE(s). The reason for the death should be reported as SAE.

For all SAEs, the investigator has to perform an assessment for seriousness and causal relationship to IMP.

7.3 Reporting of SAEs and SARs

7.3.1 Reporting responsibilities of the investigator

SAEs

All SAEs should be immediately reported by the investigator, i.e. within 24 hours after becoming aware of an event, which meets the definition of a SAE in 7.1.2. SAEs need to be reported to the sponsor's safety desk from day 1 of study treatment (= enrolment) until 28 days after last administration of study drug, regardless its causal relationship to the study drug.

If relevant information is missing at the time of the initial SAE report, the reporter should provide it in follow-up SAE report(s). Important follow-up information must also be reported as soon as they are available within 24 hours to the sponsor's safety desk. A follow-up report should contain new, updated or corrected information. The follow-up report should describe whether the event has resolved or continues, if and how it was treated including documentation of all supportive actions taken. Prompt notification (i.e. within 24h) of SAEs by the investigator to the sponsor's safety desk for SAE receipt is essential so that the legal obligations and ethical responsibilities towards the safety of the subjects are met. The investigator will comply with the applicable local regulatory requirements related to the reporting of SAE to CAs and ECs.

The investigator has to report any SAE immediately after the awareness in the AE/SAE section of the eCRF with all known and relevant information and has to sign and send the SAE page. The RELAX Safety Desk will be informed via Email about the occurrence of the SAE automatically.

In case the database does not work, the SAE has to be reported by fax or Email using the SAE form (sample in ISF).

RELAX Safety Desk, Medizinische Fakultät der TU Dresden, Fax: +49 (0)351 458 88 83518, E-mail: Safety-MK1@ukdd.de , Tel. +49 (0)351 458 5198

7.3.2 Reporting responsibilities of the sponsor

AEs

The sponsor (or its representative) has to ensure that all AEs which occurred during the course of the study are properly documented. All reported AEs will be listed and discussed in respective reports (e.g. CSR). These reports are to be submitted to the competent authorities (CAs) and/or ethics committees (ECs) according to local requirements if requested.

SAEs

The sponsor will ensure that an SAE will be reported periodically to the relevant ECs and CAs in accordance with local site/country requirements.

SUSARs

In accordance with national regulations and GCP guidelines, the sponsor should promptly notify all concerned investigators, institutions and the regulatory authorities of SUSARs and all findings that could affect adversely the safety of subjects, impact the conduct of the study or alter EC/CA approval to continue the study.

The sponsor (or its representative) has to report any reported SUSAR immediately, the latest within 7 days after notification for fatal and life-threatening SUSARs (with 8 days for follow-up), and within 15 days after notification for all other SUSARs to the relevant CAs, ECs and to the investigators.

Re-examination of the risk benefit profile

The sponsor (or its representative) has to report immediately but the latest within 15 days after s/he got aware of the occurrence of any reason that requires a re-examination of the risk benefit profile of the study medication to the relevant ethical committee and the federal authorities (BfArM).

Included are:

- case reports of expected serious adverse reactions with an unexpected outcome,
- increase of the frequency of unexpected adverse reactions that are judged as clinical relevant,
- events that are related with the clinical study or the development of the study medication that can possibly affect the safety of the participant.

List of all SARs and safety report (Development Safety Update Report)

The sponsor (or its representative) has to submit a list of SAR and a safety report to the relevant ethical committee and the federal authorities (BfArM) annually during the course of the clinical study or on request. The time frame for this report starts as soon as approval by the federal authority (BfArM) is available. The Sponsor (or its representative) will prepare these annual reports until the date of last patient last study visit, time points are defined in section 3.7.

Measures to protect against imminent danger

In case that the safety of the participants is impaired and the sponsor (or its representative) as well as the investigator take action to prevent the participants from any harm, the sponsor has to inform the relevant ethical committee and the federal authorities (BfArM) about these measures and causal circumstances.

8 Statistical analysis and sample size justification

8.1 Phase I - Dose escalation part

8.1.1 Sample size

The dose escalation part of the trial is planned as 3+3 design. In the 3+3 design the sample size is determined by the number of dosing levels and the true probability of DLTs in the dosing levels. The study is planned with 4 dosing levels including one fallback safety dosing level. In case of observing 2 DLTs in dosing level 1 and 2 DLTs in dosing level -1 the minimum number of 4 patients will be treated in the dose escalation part. In case of 1 DLT in each dosing level the maximum number of 18 patients will be treated in this study part.

8.1.2 Analysis set

8.1.2.1 MTD analysis set

The analysis set for determination of the MTD will consist of all patients who experienced a DLT and all patients who received at least the minimum of the target dose of their cohort (see section 3.2.1). Patients who did not complete DLT evaluation period due to reasons not comprising DLT will not be considered for determination of MTD and will be replaced.

8.1.2.2 Safety evaluation set (SES)

All patients with exposure to venetoclax will be part of the safety evaluation set (SES).

8.1.3 Definition of endpoint

8.1.3.1 Primary endpoint maximum tolerated dose MTD

Maximum tolerated dose of cytarabine in combination with venetoclax plus mitoxantrone in the framework of a 3+3 design is the primary endpoint. It will be determined as described in section 3.2.

Additionally, a logistic regression model logit(P(DLT)=a+b*cytarabine dose will be fitted and an estimate for the MTD is derived by calculation of (logit(p0)-a)/b. With p0 = 0.33 as the acceptable target toxicity level.

8.1.4 Statistical methods

The analysis of the phase I trial part will be of descriptive nature. It will be based on the first treatment cycle. Further treatment cycles will not be considered for determination of MTD. Changes to the analyses, specified in this protocol, will be described in a detailed statistical analysis plan.

8.1.4.1 Descriptive statistics

All study variables will be presented using appropriate descriptive statistics. For all variables the absolute numbers and percentages of non-missing and missing values will be presented. Calculated statistics for the different variable types will be as follows:

- continuous variables: arithmetic mean, standard deviation, median, interquartile range, minimum and maximum will be presented
- categorical variables: absolute number and percentage

8.1.5 Analysis of safety

Descriptive analysis of safety and toxicity will be conducted in the SES.

8.1.5.1 Extent of exposure

For each of the drugs duration of administration (number of days) and administered dose will be reported as absolute number and percentage of planned duration and dose. Number of interruptions and duration of interruptions will be reported.

8.1.5.2 Dose limiting toxicities (DLT)

All dose limiting toxicities will be listed separately per dose level, including description of the event, date of first administration of cytarabine, date of first administration of venetoclax, date of last administration of cytarabine drug, date of last administration of venetoclax, date of onset of AE, date of end of AE, severity, seriousness, relatedness to either venetoclax or cytarabine, action taken and outcome

Incidences of DLTs will be reported with counts and percentage grouped by CTCAE toxicity grade, dose level, severity, outcome, related study drug and overall.

8.1.5.3 Adverse events

Adverse events occurring before the first administration of venetoclax will be assigned to the medical history and analyzed in that context.

Incidences of AEs will be reported with counts and percentage grouped by CTCAE toxicity grade, dose level, severity, relatedness to cytarabine, relatedness to venetoclax and overall. AEs leading to discontinuation, SAEs, and deaths will be listed as described for DLTs in section 8.1.5.2.

8.1.5.4 Laboratory parameters

Laboratory parameters will be analyzed descriptively and separately for each time point. Additionally the parameters will be classified as “normal” / “abnormal, not clinically significant” / “abnormal, clinically significant” and change tables will be calculated comparing V1 with V8–EOT.

8.2 Phase II – Expansion part

8.2.1 Sample size

In combination with low-dose cytatabine, the bcl2 inhibitor venetoclax has shown promising clinical activity with a CR+CRi rate of 54% in a frail elderly patient population receiving initial treatment for newly diagnosed AML(Wei et al., J Clin Oncol 2019). In addition, cytarabine may synergize with venetoclax by down-regulating Mcl-1. In a paper from 2011, Touzeau et al. showed synergy between ABT-737 and Ara-C in mantle cell lymphoma cell lines (Touzeau et al., Clin Cancer Res 2011).

We hypothesize that the combination of intermediate-dose cytarabine salvage plus venetoclax will have an increased antileukemic efficacy compared to standard salvage alone and that efficacy signals can already be shown in a small patient number in a phase-I/II expansion cohort. Historical data show: While only around 20% of frail patients who have been treated with low –dose cytarabine alone reach CRs, the combination with venetoclax results in an increase by 30% (Burnett 2007). Standard intensive relapse treatments produce CR rates of around 40-55% (Steinmetz et al., Ann Hematol 1999; Karanes et al., Leuk Res 1999; Pastore et al., Ann Hematol 2003; Larsson et al., Leukemia & Lymphoma 2012; Fiegl et al., Leukemia 2014; Ahmed et al., Leuk Res 2015; Thiel et al., Ann Oncol 2015; Bergua et al., Br J Haematol 2016). Assuming a similar beneficial effect of venetoclax in this setting, we assume a CR rate of 65% for the combination treatment of intermediate-dose cytarabine, mitoxantrone and venetoclax.

The phase II part will be performed as single stage study according to A'Hern (A'Hern Stat Med 2001). It will test the null hypothesis that the CR/CRi rate is ≤45%. This benchmark for the CR/CRi rate was selected based on literature review of trials for this patient population. We expect a CR/CRi rate of 65% with the combination treatment as the minimum required level of efficacy. This expectation is based on CR/CRi rates reported for the combination of venetoclax and demethylating agents in patients with de novo AML being around 70% (DiNardo ASH 2015, Pollyea ASCO 2016). To be able to reject the null hypothesis (H0: $p \leq 0.45$) in favor of the alternative hypothesis (H1: $p \geq 0.65$) at a one-sided significance level of 5% with 80% power 42 patients must be enrolled.

8.2.2 Analysis sets

8.2.2.1 Full analysis set (FAS)

The FAS consists of all patients with exposure to venetoclax and cytarabine with the MTD (including patients who received MTD in phase I part, even if they were excluded from the MTD analysis set).

8.2.2.2 Per protocol set (PPS)

Consists of all patients in the FAS who received the minimum of the planned dose of venetoclax and cytarabine (see section 3.2.1) with the MTD.

8.2.2.3 Safety evaluation set (SES)

Consists of all patients with exposure to venetoclax.

8.2.3 Definition of endpoints

8.2.3.1 Primary endpoint CR/CRi rate

The CR/CRi rate is the primary endpoint of the phase II expansion part. It is determined as follows: number of patients who achieved CR or CRi, divided by the number of all patients in the analysis set.

8.2.3.2 Secondary Endpoints

- Duration of remission: Time from CR/CRi until relapse, only calculated for relapsed patients.
- Cumulative incidence of relapse: Proportion of patients in CR/CRi relapsing during observational period. Death in remission is considered as competing event. Observation is censored at the end of the observational period if no relapse occurred.
- Depth of remission (MRD): Number of patients who achieved MRD negative CR/CRi, divided by all patients with MRD marker
- Overall survival: Time from date of first application of study drug until date of death. Observation is censored at the end of the observational period in case of survival.
- Early mortality: number of patients who died within 14 and 30 days from date of first study drug administration, divided by all patients in the analysis set
- Proportion of allogeneic stem cell transplantation following response: number of patients with CR/CRi who underwent allogeneic stem cell transplantation, divided by the number of all patients who achieved CR/CRi
- Tolerability: incidence and grade of adverse events

8.2.4 Statistical methods

The primary endpoint will be analyzed confirmatory. All other analyses will be descriptive and exploratory.

8.2.4.1 Descriptive statistics

Descriptive analyses in the expansion part of the study will follow the principles as described for the phase I dose escalation study part.

8.2.4.2 Missing values

Primary endpoint: The number and proportion of missing values will be reported. Missing values can occur - for example – in the following cases:

- A patient dies before remission assessment
- A patient withdraws consent before remission assessment and does not allow collection of follow-up data
- Remission assessment is not done due to other reasons (with or without withdrawal from study treatment)
- Remission assessment is done but no valid result is available (punctio sicca, etc.)

In all cases the missing outcome is treated as failure to achieve CR/CRi. This strategy is considered to be conservative, because it can only result in underestimation of efficacy, making it harder to reject the null hypothesis.

For all other endpoints and variables the number and proportion of missing values will be reported.

Even if the patient has not been treated according to protocol or has interrupted his/her study treatment prematurely, as many endpoints as possible should be assessed. Drop-out from treatment does not mean drop-out from follow-up!

8.2.4.3 Center effects

In the primary analysis centers will not be taken into account. In a sensitivity analysis the proportion of CR/CRi is estimated using a generalized estimation equation (GEE) model with logit link function and a compound symmetry correlation structure to take into account the correlation of patients within a trial center. The model will include an intercept term only. The proportion of patients with CR/CRi will be derived by calculation of exp(b0)/(1+exp(b0)) with b0 as the estimation of the intercept term from the GEE model.

8.2.4.4 Multiplicity

The primary analysis of the primary endpoint is conducted only one time at the end of trial phase. No interim analyses are planned, no multiple comparisons are planned. Therefore no adjustment for multiple hypothesis tests is necessary. Analyses of secondary endpoints are exploratory and will therefore not be adjusted for multiplicity.

8.2.5 Analysis of Efficacy

All analyses will be conducted in the full analysis set (FAS). An analysis in the per-protocol set (PPS) is conducted as sensitivity analysis.

8.2.5.1 Primary endpoint, primary analysis

Absolute frequencies and proportion of patients who achieve CR/CRi will be calculated. A two sided 95% Clopper-Pearson confidence interval for the proportion will be calculated. The null

hypothesis (H0: p ≤ 0.45) will be tested with the exact binomial test at a one-sided significance level of 5%.

8.2.5.2 Secondary endpoints

- Duration of remission is estimated only in patients who relapse. Median and interquartile range will be presented together with the absolute number of relapsed patients and percentage based on all patients who achieved CR/CRi.
- Cumulative incidence of relapse is estimated with the competing risk methodology according to Fine and Gray (1988), death in remission is considered as competing event, cumulative incidences and two-sided 95% confidence intervals will be presented for meaningful time points.
- Depth of remission: Absolute numbers and proportion of patients with MRD negative CR/CRi and a two-sided 95% Clopper-Pearson confidence interval, separately for all planned remission control times will be presented
- Overall survival is analyzed with the Kaplan-Meier method, median overall survival time and two-sided 95% confidence interval will be estimated, proportion of surviving patients and two-sided 95% confidence intervals will be presented for meaningful time points
- Absolute numbers and proportion of patients suffering from early mortality (within 14 and 30 days) will be presented. A two-sided 95% Clopper-Pearson confidence interval for the proportion will be calculated.
- Absolute number and proportion of allogeneic stem cell transplantations following response and a two-sided 95% Clopper-Pearson confidence interval for the proportion will be calculated.

8.2.6 Exploratory endpoints

Multivariable logistic regression models will be fitted to identify biological factors predicting CR/CRi achievement, adjusted odds-ratios and two-sided 95% confidence intervals will be estimated.

8.2.7 Analysis of Safety

8.2.7.1 Extent of exposure

Extent of exposure is analyzed as described for the phase I dose escalation part of this trial (see section 8.1.5.1).

8.2.7.2 Adverse events

Analysis of adverse events will be conducted as described for the phase I dose escalation part of this trial (see section 8.1.5.3).

8.2.7.3 Laboratory parameters

Analysis of laboratory parameters will be conducted as described for the phase I dose escalation part of this trials (see section 8.1.5.4).

Additionally a comparison of V1 and EOT is done for patients who received more than one treatment cycle.

8.3 Statistical analysis plan

A detailed statistical analysis plan shall be written and finalized prior to the lock of the study database.

All statistical analyses, that were not pre-specified in the protocol or the statistical analysis plan and were conducted after database lock, will be considered as additional/exploratory analyses.

9 Data handling and quality assurance

9.1 Data recording

It is the responsibility of the investigator to perform the clinical study in accordance with the GCP guidelines, the AMG, and the clinical study protocol. All data have to be recorded correctly in the eCRF by authorized persons only. This also includes data of persons who were excluded from the clinical study.

The investigator documents the participation of a person at the Screening/Enrollment Log and Subject Identification Log. These lists are meant to identify participating patients at a later point of time. It includes the complete name, the date of birth, and the date of inclusion into the clinical study. The Subject Identification Log remains in the study center after the study is finished. In addition, participation in the clinical study has to be documented in the patient's chart (including informed consent process, screening, study medication, participant number, start and end of the clinical study).

It has to be ensured that the person responsible for documentation in the eCRF can be identified. Therefore, a list with signatures and abbreviations (site signature log) is kept in the ISF.

9.1.1 Electronic Case report form (eCRF)

All data of the participants have to be recorded in electronic CRFs.

The Investigator is responsible for all data of the participant to be documented in the electronic Case Report Form (eCRF) exclusively designed for the study immediately, correctly and completely.

Corrections in the eCRF are to be conducted only by authorized personnel and to be justified. The former database entry will remain retrievable. All dates and corrections are recorded automatically concerning date, time point and person.

9.1.2 Investigator Site File

The SAL Studienzentrale provides the Investigator Site File to the study center. The ISF includes all documents that are required for the clinical study. During monitoring, the ISF will be checked regularly for completeness and actuality. After the clinical trial is finished or stopped, the ISF has to be stored for at least 10 years.

9.1.3 Data Storage

Responsibilities of the Sponsor
As required by law, all important study documents have to be stored by the sponsor for at least 10 years after the clinical trial was finished or stopped.

Responsibilities of the Investigator

All documents which are related to the clinical study and to the distribution of the study medication (e.g. CRFs, written informed consent forms, study medication lists and other relevant material) have to be stored for at least 10 years.

Source data like patients' charts, laboratory analyzes, and other original data have to be stored for the longest possible time that is usual practice at the investigator's site, at least for 10 years.

9.2 Monitoring

The investigator agrees that the monitor will visit the study center in appropriate intervals. During these visits, the monitor will check the quality of the data recording and ensure that the study center adheres to the timeframe as set in the study protocol. The investigators agree to provide any relevant information and documentation whenever requested by the monitor. This includes access to all original study documents and source data including access to electronic source documents if necessary.

It is the responsibility of the investigator to keep the participant's chart as complete as possible (e.g. history, concomitant diseases, inclusion in the clinical study, visit dates, results of laboratory tests, distribution of the study medication, and adverse events). Source data are checked and compared with entries in the database. The participant has given consent with this procedure by signing the patient information and written informed consent form.

Additional tasks of the monitor are:

- To check, whether the study center fulfills requirements of the clinical study (e.g. participant population, technical equipment, adequate storage of medication),

- Instruction of the investigators and personnel for the clinical trial,
- To check the ISF for completeness and actuality,
- Documentation of the status of the participant,
- Matching of original data,
- To check SAE reports according to regulations.

The monitor has the responsibility to treat all information confidentially and to safeguard the integrity and personal privacy of the study participants.

9.3 Data processing

Data management is performed by the Data Center of the SAL Studienzentrale and the KKS Dresden by means current version of study software MACRO. Data quality will be checked by programmed range checks, validity checks, and consistency checks. In addition, a manual/visual data check for medicinal plausibility is done according to GCP guidelines. There might be discrepancies, which are to be clarified by authorized persons by means of the study software.

After the study is finished and before data are analyzed, a meeting will be held between the sponsor and statistician. When the database has been declared to be complete and accurate, it will be locked. This procedure has to be documented.

9.4 Audit and inspection

To guarantee the conduct of the clinical study according to GCP guidelines, sponsor audits and governmental inspections can be performed.

During the audits and inspections, the following aspects are checked:

- Conduct of the study according to the protocol,
- Data validity, patient safety
- Quality of the study according to GCP guidelines.

10 Premature termination of the study

The sponsor has the right to close this study (or, if applicable, individual segments thereof [e.g. study arms, dose steps, centers]) at any time, which may be due but not limited to the following reasons:

- If risk-benefit ratio becomes unacceptable owing to, for example, safety findings from this study (e.g. SAEs)
- Results of any interim analysis
- Recommendations from steering committee
- New scientific discoveries from literature or results from other clinical studies

- Results of parallel animal studies (on e.g. toxicity, teratogenicity, carcinogenicity, or reproduction toxicity).
- If the study conduct (e.g. recruitment rate, dropout rate, data quality, protocol compliance) does not suggest a proper completion of the study within a reasonable period.

The investigator has the right to close his / her center at any time.

For any of the above closures, the following applies:

- Closures should occur only after consultation between involved parties.
- All affected institutions (e.g. IEC(s) / IRB(s); competent authority(ies); study center(s)) must be informed as applicable according to local law.
- All study materials (except documentation that has to remain stored at site) must be returned to the sponsor. The investigator will retain all other documents until notification given by the sponsor for destruction.
- In case of a partial study closure, ongoing subjects, including those in post-study follow-up, must be taken care of in an ethical manner.

Details for individual subject's withdrawal can be found in section 4.4.

11 Ethical and legal aspects

11.1 Ethical and legal conduct of the study

The procedures set out in this protocol, about the conduct, evaluation, and documentation of this study, are designed to ensure that the sponsor and investigator abide by GCP Guidelines and under the guiding principles detailed in the Declaration of Helsinki. The study will also be carried out in keeping with applicable local law(s) and regulation(s).

Strict adherence to all specifications laid down in this protocol is required for all aspects of study conduct; the investigator may not modify or alter the procedures described in this protocol.

Modifications to the study protocol will not be implemented by either the sponsor or the Coordinating investigator without agreement by both parties. However, the investigator or the sponsor may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to the study subjects without prior IEC / IRB / sponsor approval / favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it and if appropriate the proposed protocol amendment should be submitted to the IEC / IRB / sponsor. Any deviations from the protocol must be explained and documented by the investigator.

11.2 Subject information and consent

Before inclusion in the clinical study, the investigator or its qualified delegate physician must explain to each patient the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved, and any discomfort it may entail.

Each patient must be informed that participation in the study is voluntary and that she/he may withdraw from the study at any time and that withdrawal of consent will not affect her/his subsequent medical treatment or relationship with the treating physician.

This informed consent should be given by means of a standard written statement, written in nontechnical German language. The patient should read and consider the statement before signing and dating it. If written consent is not possible, oral consent can be obtained if witnessed by a signed statement from one or more persons not involved in the study, mentioning why the patient was unable to sign the form. No patient can enter the study before his/her informed consent has been obtained.

The informed consent form is signed and dated by the participant and by the investigator. The original is kept in the ISF and a copy is given to the participant.

Any changes to the proposed consent form suggested by the investigator must be agreed to by the sponsor before submission to the IEC, and a copy of the approved version must be provided to the monitor after IEC approval.

11.3 Participants' insurance

On behalf of the sponsor, in accordance with § 40 (1) no. 8 and (3) AMG the prescribed participants' insurance was obtained at the following company:

Name:	CNA Insurance Company Limited
Insurance No.:	10306398
Address:	Im Mediapark 8, 50670 Köln
Tel.:	0221/9499-860
Fax:	0221/9499-8699

Cover extends to health impairments resulting from drugs and/or substances/investigational products administered in the course of the clinical trial for which the participant has given his/her written informed consent to participate.

Cover also extends to health impairments through measures carried out on the body of the person in connection with the clinical trial of a drug and/or substance/investigational product carried out in accordance with the study protocol procedures.

Cover extends to maximum 500.000 €.

In order to ensure the cover, the study participants have to follow strictly the instructions of the study team. They are not allowed to undergo any other medical treatment without consent of the investigators (except emergencies).

In case of an emergency treatment they have to inform the investigator as soon as possible.

A health impairment that could possibly result from the study treatment has to be announced immediately to the investigator and to the insurance company.

Furthermore, the participants are forced to take all appropriate measures to clarify cause and extensiveness of the damage occurred.

The conditions of the insurance are to be handed out to the participant together with a copy of the signed informed consent.

11.4 Publication policy

The sponsor is interested in the publication of the results of every study it performs. All relevant aspects regarding publication will be part of the contract between the sponsor and the investigator / institution.

The sponsor has committed to the global industry position on disclosure of information about clinical studies. The information regarding the study protocol is made publicly available on the internet at www.clinicaltrials.gov. This derives from the standards that international medical journal editors have established requiring protocol registration at the outset of the study as a prerequisite of consideration for publication.

11.5 Privacy and confidentiality

Recording, storage, disclosure, and analysis of personal data of the participants within this clinical study are in accordance with legal requirements (Sächsisches Datenschutzgesetz, Bundesdatenschutzgesetz, EU data protection regulation 2018). The participant has to agree on the handling of his/her data within the informed consent form. The participant has to be informed about:

- Data are recorded electronically in eCRFs, will be handled confidentially, and disclosed to others (sponsor, local and federal authorities, independent ethical committee, European data base) only pseudonymized.
- Persons who are authorized by the sponsor and the authorities to monitor and inspect the clinical study can have insight into participant related data. These persons have to handle the data confidentially. The clinical investigator is dispensed from his/her medical confidentiality towards these persons.

- The written consent for data recording and documentation during this clinical study is irreversible. When a participant withdraws the written consent, all data which are documented so far can be used in a pseudononymized way to analyze the effect of the study medication if needed.

12 Amendments

Any change or addition to this protocol requires a written protocol amendment which must be approved and signed by the sponsor and the investigator before implementation. Amendments significantly affecting the safety of patients, the scope of the investigation, or the scientific quality of the study require additional approval by the IEC, and by the federal regulatory authority (BfArM). A copy of the written approval of the IEC and CA must be given to the monitor. Examples of amendments requiring such approval are:

1. An increase in drug dosage or duration of exposure of patients,
2. A significant change in the study design (e.g., addition or deletion of a control group, inclusion/exclusion criteria),
3. An increase in the number of invasive procedures to which patients are exposed,
4. Addition or deletion of a test procedure for safety monitoring.

These requirements for approval should in no way prevent any immediate action from being taken by the investigator or by the sponsor in the interests of preserving the safety of all patients included in the trial. If an immediate change to the protocol is felt to be necessary by the investigator and is implemented by him/her for safety reasons, the sponsor should be notified and the IEC at the center should be informed within 10 working days. Actions against immediate danger needs to be reported **immediately** to the CA, local authority, and EC (GCP-V §13 (5).

Amendments affecting only administrative aspects of the study do not require formal protocol amendments or IEC approval, but the IEC must be kept informed of such administrative changes. Examples of administrative changes not requiring formal protocol amendments and IEC approval include:

1. Changes in the staff used to monitor trials (e.g., Sponsor staff versus a CRO),
2. Minor changes in the packaging or labeling of study drug.

13 Signatures and agreement with the protocol

We, the undersigned, agree to conduct this study according to the above protocol and to make no additions or changes without the consent of the sponsor. In addition, we agree that the trial will be carried out in accordance with Good Clinical Practice (GCP), with the Declaration of Helsinki and with the laws and regulations of the country in which the study takes place.

Coordinating Investigator on behalf of the Sponsor

PD Dr. med. Christoph Röllig, M.Sc.

20.09.2019

Date, signature

Biometrics

Dipl.-Psych. Michael Kramer, M.Sc.

20.09.2019

Date, signature

Investigator/s

We, the undersigned, agree to conduct this study according to the above protocol. We commit ourselves to treat, to follow-up, and to document all included participants according to the study protocol.

Last name, first name (block letters)

Date, signature

Address (Stamp):

This protocol has been designed in consideration of ICH-GCP.

14 References

A'Hern RP. Sample size tables for exact single-stage phase II designs. Stat Med. 2001 Mar 30;20(6):859-66.

Ahmed T, Holwerda S, Klepin HD, Isom S, Ellis LR, Lyerly S, Manuel M, Dralle S, Berenzon D, Powell BL, Pardee TS. High dose cytarabine, mitoxantrone and l-asparaginase (HAMA) salvage for relapsed or refractory acute myeloid leukemia (AML) in the elderly. Leuk Res. 2015 Sep;39(9):945-9.

Amadori S, Arcese W, Isacchi G, Meloni G, Petti MC, Monarca B, Testi AM, Mandelli F. Mitoxantrone, etoposide, and intermediate-dose cytarabine: an effective and tolerable regimen for the treatment of refractory acute myeloid leukemia. J Clin Oncol. 1991 Jul;9(7):1210-4.

Bergua JM, Montesinos P, Martinez-Cuadrón D, Fernández-Abellán P, Serrano J, Sayas MJ, Prieto-Fernandez J, García R, García-Huerta AJ, Barrios M, Benavente C, Pérez-Encinas M, Simiele A, Rodríguez-Macias G, Herrera-Puente P, Rodríguez-Veiga R, Martínez-Sánchez MP, Amador-Barciela ML, Riaza-Grau R, Sanz MA; PETHEMA group. A prognostic model for survival after salvage treatment with FLAG-Ida +/- gemtuzumab-ozogamicine in adult patients with refractory/relapsed acute myeloid leukaemia. Br J Haematol. 2016 Apr 26.

Büchner T, Berdel WE, Schoch C, Haferlach T, Serve HL, Kienast J, Schnittger S, Kern W, Tchinda J, Reichle A, Lengfelder E, Staib P, Ludwig WD, Aul C, Eimermacher H, Balleisen L, Sauerland MC, Heinecke A, Wörmann B, Hiddemann W. Double induction containing either two courses or one course of high-dose cytarabine plus mitoxantrone and postremission therapy by either autologous stem-cell transplantation or by prolonged maintenance for acute myeloid leukemia. J Clin Oncol. 2006 Jun 1;24(16):2480-9.

Burnett AK, Milligan D, Prentice AG, Goldstone AH, McMullin MF, Hills RK, Wheatley K. A comparison of low-dose cytarabine and hydroxyurea with or without all-trans retinoic acid for acute myeloid leukemia and high-risk myelodysplastic syndrome in patients not considered fit for intensive treatment. Cancer. 2007 Mar 15;109(6):1114-24.

Cairo MS, Bishop M. Tumour lysis syndrome: new therapeutic strategies and classification. Br J Haematol. 2004;127(1):3-11.

Coiffier B, Altman A, Pui CH, et al. Guidelines for the management of pediatric and adult tumor lysis syndrome: an evidence-based review. J Clin Oncol. 2008;26(16):2767-78.

Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, Dombret H, Ebert BL, Fenaux P, Larson RA, Levine RL, Lo-Coco F, Naoe T, Niederwieser D, Ossenkoppele GJ, Sanz M, Sierra J, Tallman MS, Tien HF, Wei AH, Löwenberg B, Bloomfield CD. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017 Jan 26;129(4):424-447.

Fesik SW. Promoting apoptosis as a strategy for cancer drug discovery. Nat Rev Cancer. 2005;5(11):876-85.

Fiegl M, Unterhalt M, Kern W, Braess J, Spiekermann K, Staib P, Grüneisen A, Wörmann B, Schöndube D, Serve H, Reichle A, Hentrich M, Schiel X, Sauerland C, Heinecke A, Rieger C, Beelen D, Berdel WE, Büchner T, Hiddemann W; German AML Cooperative Group (AMLCG). Chemomodulation of sequential high-dose cytarabine by fludarabine in relapsed or refractory acute myeloid leukemia: a randomized trial of the AMLCG. Leukemia. 2014 May;28(5):1001-7.

Jeziskova I, Musilova M, Culen M, Foltankova V, Dvorakova D, Mayer J, Racil Z. Distribution of mutations in DNMT3A gene and the suitability of mutations in R882 codon for MRD monitoring in patients with AML. Int J Hematol. 2015 Nov;102(5):553-7.

Ji Y, Wang SJ. Modified toxicity probability interval design: a safer and more reliable method than the 3 + 3 design for practical phase I trials. J Clin Oncol. 2013 May 10;31(14):1785-91.

Karanes C, Kopecky KJ, Head DR, Grever MR, Hynes HE, Kraut EH, Vial RH, Lichtin A, Nand S, Samlowski WE, Appelbaum FR. A phase III comparison of high dose ARA-C (HIDAC) versus HIDAC plus mitoxantrone in the treatment of first relapsed or refractory acute myeloid leukemia Southwest Oncology Group Study. Leuk Res. 1999 Sep;23(9):787-94.

Larson SM, Campbell NP, Huo D, Artz A, Zhang Y, Gajria D, Green M, Weiner H, Daugherty C, Odenike O, Godley LA, Hyjek E, Gurbuxani S, Thirman M, Sipkins D, Van Besien K, Larson RA, Stock W. High dose cytarabine and mitoxantrone: an effective induction regimen for high-risk acute myeloid leukemia (AML). Leuk Lymphoma. 2012 Mar;53(3):445-50.

Ossenkoppele GJ, Graveland WJ, Sonneveld P, Daenen SM, Biesma DH, Verdonck LF, Schaafsma MR, Westveer PH, Peters GJ, Noordhuis P, Muus P, Selleslag D, van der Holt B, Delforge M, Löwenberg B, Verhoef GE; Dutch-Belgian Hemato-Oncology Cooperative Group (HOVON). The value of fludarabine in addition to ARA-C and G-CSF in the treatment of patients with high-risk myelodysplastic syndromes and AML in elderly patients. Blood. 2004 Apr 15;103(8):2908-13.

Pastore D, Specchia G, Carluccio P, Liso A, Mestice A, Rizzi R, Greco G, Buquicchio C, Liso V. FLAG-IDA in the treatment of refractory/relapsed acute myeloid leukemia: single-center experience. Ann Hematol. 2003 Apr;82(4):231-5.

Scatizzi JC, Hutcheson J, Pope RM, et al. Bim-BCL-2 homology 3 mimetic therapy is effective at suppressing inflammatory arthritis through the activation of myeloid cell apoptosis. Arthritis Rheum. 2010;62(2):441-51.

Shlush LI, Zandi S, Mitchell A, Chen WC, Brandwein JM, Gupta V, Kennedy JA, Schimmer AD, Schuh AC, Yee KW, McLeod JL, Doedens M, Medeiros JJ, Marke R, Kim HJ, Lee K, McPherson JD, Hudson TJ; HALT Pan-Leukemia Gene Panel Consortium, Brown AM, Yousif F, Trinh QM, Stein LD, Minden MD, Wang JC, Dick JE. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. Nature. 2014 Feb 20;506(7488):328-33.

Steinmetz HT, Schulz A, Staib P, Scheid C, Glasmacher A, Neufang A, Franklin J, Tesch H, Diehl V, Dias Wickramanayake P. Phase-II trial of idarubicin, fludarabine, cytosine arabinoside, and

filgrastim (Ida-FLAG) for treatment of refractory, relapsed, and secondary AML. Ann Hematol. 1999 Sep;78(9):418-25.

Thiel A, Schetelig J, Pönisch W, Schäfer-Eckart K, Aulitzky W, Peter N, Schulze A, Maschmeyer G, Neugebauer S, Herbst R, Hänel A, Morgner A, Kroschinsky F, Bornhäuser M, Lange T, Wilhelm M, Niederwieser D, Ehninger G, Fiedler F, Hänel M; East German Study Group Hematology/Oncology (OSHO); Study Alliance Leukemia (SAL). Mito-FLAG with Ara-C as bolus versus continuous infusion in recurrent or refractory AML--long-term results of a prospective randomized intergroup study of the East German Study Group Hematology/Oncology (OSHO) and the Study Alliance Leukemia (SAL). Ann Oncol. 2015 Jul;26(7):1434-40.

Thol F, Schlenk RF, Heuser M, Ganser A. How I treat refractory and early relapsed acute myeloid leukemia. Blood. 2015 Jul 16;126(3):319-27.

Tischner D, Woess C, Ottina E, et al. BCL-2-regulated cell death signaling in the prevention of autoimmunity. Cell Death Dis. 2010;1:e48.

Touzeau C, Dousset C, Bodet L, Gomez-Bougie P, Bonnaud S, Moreau A, Moreau P, PellatDeceunynk C, Amiot M, Le Gouill S. ABT-737 induces apoptosis in mantle cell lymphoma cells with a BCL-2high/Mcl-1low profile and synergizes with other antineoplastic agents. Clin Cancer Res. 2011 Sep 15;17(18):5973-81.

Wei A, Tiong I, Roberts A, Fong C et al. Chemotherapy and venetoclax in elderly AML trial (CAVEAT): A phase 1b dose escalation study examining modified intensive chemotherapy in fit elderly patients. Annual Meeting of the EHA 2018, abstract S1564.

Wei A, Strickland SA, Roboz GJ et al. Safety and Efficacy of Venetoclax Plus Low-Dose Cytarabine in Treatment-Naive Patients Aged ≥65 Years with Acute Myeloid Leukemia. Blood 2016;128:102

Wei AH, Strickland SA Jr, Hou JZ, Fiedler W, Lin TL, Walter RB, Enjeti A, Tiong IS, Savona M, Lee S, Chyla B, Popovic R, Salem AH, Agarwal S, Xu T, Fakouhi KM, Humerickhouse R, Hong WJ, Hayslip J, Roboz GJ. Venetoclax Combined With Low-Dose Cytarabine for Previously Untreated Patients With Acute Myeloid Leukemia: Results From a Phase Ib/II Study. J Clin Oncol. 2019 May 20;37(15):1277-1284.

15 Appendices

Appendix 1

Sample List of Excluded and Cautionary Medications

Excluded or causing dose reduction of venetoclax
Strong CYP3A inhibitors boceprevir, clarithromycin, conivaptan, indinavir, itraconazole, ketoconazole, lopinavir/ritonavir, mibefradil, nefazodone, nelfinavir, posaconazole, ritonavir, saquinavir, telaprevir, telithromycin, voriconazole **Strong CYP3A inducers** avasimibe, carbamazepine (Tegretol®), phenytoin (Dilantin®), rifampin (Rifadin®), St. John's wort **Moderate CYP3A inhibitors** amprenavir, aprepitant, atazanavir, ciprofloxacin, crizotinib*, darunavir/ritonavir, diltiazem, erythromycin, fluconazole, fosamprenavir, imatinib*, verapamil **Moderate CYP3A inducers** bosentan, efavirenz, etravirine, modafinil, nafcillin
Cautionary
Warfarin ** **P-gp substrates** Aliskiren, ambrisentan, colchicine, dabigatran etexilate, digoxin, everolimus*, fexofenadine, lapatinib*, loperamide, maraviroc, nilotinib*, ranolazine, saxagliptin, sirolimus*, sitagliptin, talinolol, tolvaptan, topotecan* **BCRP substrates** Methotrexate*, mitoxantrone*, irinotecan*, lapatinib*, rosuvastatin, sulfasalazine, topotecan* **OATP1B1/1B3 substrates** Atrasentan, atorvastatin, ezetimibe, fluvastatin, glyburide, rosuvastatin, pitavastatin, pravastatin, repaglinide, simvastatin acid, telmisartan, valsartan, olmesartan **P-gp inhibitors** Amiodarone, azithromycin, captopril, carvedilol, dronedarone, felodipine, quercetin, ronalzine, ticagrelor **BCRP inhibitors** Cyclosporine* geftinib*

Note that this is not an exhaustive list. For an updated list, see the following link: https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm080499.htm

In addition to the medications listed in this table, subjects receiving venetoclax should not consume grapefruit, grapefruit products, Seville oranges (including marmalade containing Seville oranges) or Starfruits.

* These are anticancer agents; consult contact the AbbVie medical monitor before use.

**Closely monitor the international normalized ratio (INR).

Appendix 2

Recommendations for Initial Management of Electrolyte Imbalances and Prevention of Tumor Lysis Syndrome (TLS)

Abnormality	Management Recommendations
Hyperkalemia (including rapidly rising potassium)	
Potassium ≥ 0.5 mmol/L increase from prior value (even if potassium within normal limits [WNL])	• Recheck potassium, phosphorus, uric acid, calcium and creatinine in 1 hour STAT. If further ≥ 0.2 mmol/L increase in potassium, but still < upper limit of normal (ULN), manage as per potassium ≥ ULN. Otherwise recheck in 1 hour. • Resume per protocol testing if change in potassium is < 0.2 mmol/L, and potassium < ULN, and no other evidence of tumor lysis. • At discretion of investigator, may recheck prior to hospitalization. If stable or decreased, and still WNL, hospitalization is at the discretion of the investigator. Potassium, phosphorus, uric acid, calcium and creatinine must be rechecked within 24 hours.
Potassium > upper limit of normal	• Perform STAT ECG and commence telemetry. • Nephrology (or other acute dialysis service) notification with consideration of initiating dialysis. • Administer Kayexalate 60 g (or Resonium A 60 g). • Administer furosemide 20 mg IV × 1. • Administer calcium gluconate 100 – 200 mg/kg IV slowly if there is ECG/telemetry evidence of life-threatening arrhythmias. • Recheck potassium, phosphorus, uric acid, calcium and creatinine in 1 hour STAT. • If potassium < ULN 1 hour later, repeat potassium, phosphorus, uric acid, calcium and creatinine 1, 2 and 4 hours, if no other evidence of tumor lysis.
Potassium ≥ 6.0 mmol/L (6.0 mEq/L) and/or symptomatic (e.g., muscle cramps, weakness, paresthesias, nausea, vomiting, diarrhea)	• Perform STAT ECG and commence telemetry. • Nephrology (or other acute dialysis service) assessment with consideration of initiating dialysis. • Administer Kayexalate 60 g (or Resonium A 60 g). • Administer furosemide 20 mg IV × 1. • Administer insulin 0.1 U/kg IV + D25 2 mL/kg IV. • Administer sodium bicarbonate 1 – 2 mEq/kg IV push. • If sodium bicarbonate is used, rasburicase should not be used as this may exacerbate calcium phosphate precipitation. • Administer calcium gluconate 100 – 200 mg/kg IV slowly if there is ECG/telemetry evidence of life-threatening arrhythmias. Do not administer in same IV line as sodium bicarbonate. • Recheck potassium, phosphorus, uric acid, calcium and creatinine every hour STAT.

Abnormality	Management Recommendations
Hyperuricemia	
Uric acid ≥ 8.0 mg/dL (476 µmol/L)	• Consider rasburicase (dose per institutional guidelines). • If rasburicase is used, sodium bicarbonate should not be used as this may exacerbate calcium phosphate precipitation. • Recheck potassium, phosphorus, uric acid, calcium and creatinine in 1 hour STAT.
Uric acid ≥ 10 mg/dL (595 µmol/L) OR Uric acid ≥ 8.0 mg/dL (476 µmol/L) with 25% increase and creatinine increase ≥ 0.3 mg/dL (≥ 0.027 mmol/L) from pre-dose level	• Administer rasburicase (dose per institutional guidelines). • If rasburicase is used, sodium bicarbonate should not be used as this may exacerbate calcium phosphate precipitation. • Notify nephrology (or other acute dialysis service). • Recheck potassium, phosphorus, uric acid, calcium and creatinine in 1 hour STAT. • If uric acid < 8.0 mg/dL 1 hour later, repeat potassium, phosphorus, uric acid, calcium and creatinine 2 and 4 hours later, if no other evidence of tumor lysis.
Hypocalcemia	
Calcium ≤ 7.0 mg/dL (1.75 mmol/L) AND Patient symptomatic e.g., muscle cramps, hypotension, tetany, cardiac arrhythmias)	• Administer calcium gluconate 50 – 100 mg/kg IV slowly with ECG monitoring. • Telemetry. • Recheck potassium, phosphorus, uric acid, calcium and creatinine in 1 hour STAT. • If calcium normalized 1 hour later, repeat potassium, phosphorus, uric acid, calcium and creatinine 2 and 4 hours later, if no other evidence of tumor lysis. Calculate corrected calcium and check ionized calcium if albumin low.
Hyperphosphatemia	
Phosphorus ≥ 5.0 mg/dL (1.615 mmol/L) with ≥ 0.5 mg/dL (0.16 mmol/L) increase	• Administer a phosphate binder (e.g., aluminum hydroxide, calcium carbonate, sevelamer hydroxide, or lanthanum carbonate). • Nephrology (or other acute dialysis service) notification (dialysis required for phosphorus ≥ 10 mg/dL). • Re-check potassium, phosphorus, uric acid, calcium and creatinine in 1 hour STAT. If phosphorus < 5.0 mg/dL 1 hour later, repeat potassium, phosphorus, uric acid, calcium and creatinine 2 and 4 hours later, if no other evidence of tumor lysis.
Creatinine	
Increase ≥ 25% from baseline	• Start or increase rate of IV fluids. • Recheck potassium, phosphorus, uric acid, calcium and creatinine in 1 – 2 hours STAT.

Appendix 3

Definitions of Laboratory and Clinical Tumor Lysis Syndrome*

Metabolic Abnormality	Criteria for Classification of Laboratory Tumor Lysis Syndrome	Criteria for Classification of Clinical Tumor Lysis Syndrome
Hyperruricemia	Uric acid > 8.0 mg/dl (475.8 µmol/liter) in adults or above the upper limit of the normal range for age in children	
Hyperphosphatemia	Phosphorus > 4.5 mg/dl (1.5 mmol/liter) in adults or > 6.5 mg/dl (2.1 mmol/liter) in children	
Hyperkalemia	Potassium > 6.0 mmol/liter	Cardiac dysrhythmia or sudden death probably or definitely caused by hyperkalemia
Hypocalcemia	Corrected calcium < 7.0 mg/dl (1.75 mmol/liter) or ionized calcium < 1.12 (0.3 mmol/liter)†	Cardiac dysrhythmia, sudden death, seizure, neuromuscular irritability (tetany, paresthesias, muscle twitching, carpopedal spasm, Trousseau's sign, Chvostek's sign, laryngospasm, or bronchospasm), hypotension, or heart failure probably or definitely caused by hypocalcemia
Acute kidney injury‡	Not applicable	Increase in the serum creatinine level of 0.3 mg/dl (26.5 µmol/liter) (or a single value > 1.5 times the upper limit of the age-appropriate normal range if no baseline creatinine measurement is available) or the presence of oliguria, defined as an average urine output < 0.5 mL/kg/hr for 6 hrs

† The corrected calcium level in milligrams per deciliter = measured calcium level in milligrams per deciliter + 0.8 × (4-albumin in grams per deciliter).

‡ Acute kidney injury is defined as an increase in the creatinine level of at least 0.3 mg per deciliter (26.5 µmol per liter) or a period of oliguia lasting 6 hours or more. By definition, if acute kidney injury is present, the patient has clinical tumor lysis syndrome.

Note: In laboratory tumor lysis syndrome, two or more metabolic abnormalities must be present during the same 24-hour period within 3 days before the start of therapy or up to 7 afterward. Clinical tumor lysis syndrome requires the presence of laboratory tumor lysis syndrome plus an increased creatinine level, seizures, cardiac dysrhythmia, or death.

* Howard SC, Jones DP, Pui CH. The tumor lysis syndrome. N Engl J Med. 011;364(19):1844-54.

Appendix 4

Clinical Laboratory Tests

Hematology	Clinical Chemistry[a]	Urinalysis
Hematocrit Hemoglobin Red blood cell (RBC) count White blood cell (WBC) count Microscopic differential: Neutrophils Bands (if detected) Lymphocytes Monocytes Basophils (if detected) Eosinophils (if detected) Platelet count (estimate not acceptable) Blasts	Urea Creatinine Calculated or Measured creatinine clearance Total bilirubin Serum glutamic-pyruvic transaminase (SGPT/ALT) Serum glutamic-oxaloacetic transaminase (SGOT/AST) Alkaline phosphatase Sodium Potassium Calcium Inorganic phosphorus Uric acid[b] Total protein Glucose Albumin Lactate dehydrogenase (LDH) Magnesium Chloride Amylase Lipase ß-HCG i.S./P. oder SST i.U.	Specific gravity Ketones Ph Protein Blood Glucose Microscopic examination (as indicated)
Coagulation		
Prothrombin time (PT) Activated partial thromboplastin time (aPTT) INR Fibrinogen		

a. All chemistries should be performed at all visits except at TLS time points during dose ramp up (i.e., the first week of therapy) or when otherwise noted.

b. At room temperature, rasburicase causes enzymatic degradation of the uric acid in blood/plasma/serum samples potentially resulting in spuriously low plasma uric acid assay readings. The following special sample handling procedure must be followed to avoid ex vivo uric acid degradation.

Uric acid must be analyzed in plasma. Blood must be collected into pre-chilled tubes containing heparin anticoagulant. **Immediately immerse plasma samples for uric acid measurement in an ice water bath**. Plasma samples must be prepared by centrifugation in a pre-cooled centrifuge (4°C). Finally, the plasma must be maintained in an ice water bath and analyzed for uric acid within 4 hours of collection.

Appendix 5

Adverse Events Commonly Associated with AML Study Population and/or Progression of AML

PreferredTerm(MedDRAVersion16.1)

- Fever
- Fatigue
- Dyspnea
- Pain, all types
- Thrombocytopenia
- Anemia Neutropenia
- Infection (bacterial, viral, fungal)
  - Neutropenic infection
  - Neutropenic sepsis
  - Oral candidiasis
  - Stomatitis
  - Periodontal infection
  - Tooth infection, abscess
  - Upper respiratory tract infection
  - Sinusitis, Rhinitis
  - Bronchitis (bacterial, viral) Bronchitis chronic
  - Pneumonia (bacterial, viral, fungal) Catheter site cellulitis
  - Herpes zoster disseminated, multi-dermatomal
  - Herpes zoster
  - Herpes simplex (oral, genital)
  - Skin candida
  - Urinary tract infection bacterial, fungal
  - Genitourinary tract infection (viral, bacterial, fungal)
  - Gastroenteritis
  - Enterocolitis
- Malignant disease progression, including death
  - Hyperleukocytosis, including the symptomatic form (leukostasis)
  - Leukaemic infiltration brain
  - Malignant pleural effusion
  - Chloroma
- Second primary cancers, all types
- Bleeding
  - Gingival bleeding
  - Mouth haemorrhage
  - Epistaxis
  - Haematuria
  - Injection site haemorrhage
  - Petechiae
  - Skin haemorrhage
  - Retinal hemorrhage

Appendix 6

WHO Classification of AML *

Sub Group	Spezifikation
Acute Myeloid Leukemia with recurrent genetic aberrations	AML with t(8;21)(q22;q22); RUNX1-RUNX1T1
	AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11
	APL with t(15;17)(q22;q12); PML-RARA
	AML with t(9;11)(p22;q23); MLLT3-KMT2A
	AML with t(6;9)(p23;q34); DEK-NUP214
	AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); GATA2, MECOM
	AML (megakaryoblastic) with t(1;22)(p13;q13); RBM15-MKL1
	Provisional entity: AML with BCR-ABL1
	AML with mutated NPM1
	AML with biallelic mutations of CEBPA
	Provisional entity: AML with mutated RUNX1
Acute myeloid leukemia with myelodysplasia-related changes	
Therapy-related myeloid neoplasms	
Acute myeloid leukemia, not otherwise specified (NOS)	Acute myeloid leukemia with minimal differentiation
	Acute myeloid leukemia without maturation
	Acute myeloid leukemia with maturation
	Acute myelomonocytic leukemia
	Acute monoblastic/monocytic leukemia
	Pure erythroid leukemia
	Erythroleukemia, erythroid/myeloid
	Acute megakaryoblastic leukemia
	Acute basophilic leukemia
	Acute panmyelosis with myelofibrosis (syn.: acute myelofibrosis; acute myelosclerosis)
Myeloid sarcoma	
Myeloid proliferations related to Down-syndrome	Myeloid leukemia associated with Down syndrome
	Transient abnormal myelopoiesis (syn.: transient myeloproliferative disorder)
Acute leukemias of ambiguous lineage	Acute undifferentiated leukemia
	Mixed phenotype acute leukemia with t(9;22)(q34;q11.2); BCR-ABL1
	Mixed phenotype acute leukemia with t(v;11q23); MLL rearranged/KMT2A
	Mixed phenotype acute leukemia, B/myeloid, NOS
	Mixed phenotype acute leukemia, T/myeloid, NOS

*Arber DA, Orazi A, Hasserjian R et al.: The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 127:2391-2405, 2016

Appendix 7

Molecular-zytogenetic Risk groups according Classification of European LeukemiaNet ELN [#]

ELN Risikogruppe	Aberrationen
Günstig	• t(8;21)(q22;q22); *RUNX1-RUNX1T1* • inv(16)(p13.1q22) oder t(16;16)(p13.1;q22); *CBFB-MYH11* • Mutiertes *NPM1* ohne *FLT3*-ITD oder mit FLT3-ITD^{niedrig*} • Biallelisch mutiertes *CEBPA*
intermediär	• Mutiertes *NPM1* mit *FLT3*-ITD^{hoch*} (normaler Karyotyp) • Wildtyp-*NPM1* ohne *FLT3*-ITD (normaler Karyotyp) oder mit FLT3-ITD^{niedrig*} (mit oder ohne ungünstige genetische Aberrationen) • t(9;11)(p22;q23); *MLLT3-KMT2A*[§] • Zytogenetische Aberrationen, die nicht als günstig oder ungünstig eingestuft wurden
Ungünstig	• t(6;9)(p23;q34); *DEK-NUP214* • t(v;11)(v;q23); *KMT2A*-Genumlagerung • t(9;22)(q34.1;q11.2); *BCR-ABL1* • inv(3)(q21q26.2) oder t(3;3)(q21;q26.2); GATA2, MECOM (*EVI1)* • -5 oder del(5q); -7; -17/abnl(17p) • komplexer Karyotyp (≥3 Aberrationen[†]) • monosomaler Karyotyp (eine Monosomie, assoziiert mit mindestens einer weiteren Monosomie oder einer anderen strukturellen, chromosomalen Aberration (außer CBF-AML)) • Wildtyp-*NPM1* mit *FLT3*-ITD^{hoch*} • Mutiertes *RUNX1*[‡] • Mutiertes *ASXL1*[‡] • Mutiertes *TP53*

Legende:

* FLT3-ITDniedrig = Mutant-Wildtyp-Allel-Quotient <0,5; FLT3-ITDhoch = Mutant-Wildtyp-Allel-Quotient ≥0,5. Bestimmung über semi-quantitative Messung des FLT3-ITD Allel-Quotienten mittels DNA-Fragment-Analyse als Quotient der AUC für FLT3-ITD dividiert durch die AUC für FLT3-Wildtyp

§ in Anwesenheit seltenerer als ungünstig eingestufter Aberrationen „sticht" die t(9;11), d.h. sie gibt den Ausschlag für eine Einstufung in die intermediäre Risikogruppe

† nur zutreffend, wenn nicht gleichzeitig eine der WHO-definierten AML-typischen Aberrationen vorliegt (d.h. t(8;21), inv(16) oder t(16;16), t(9;11), t(v;11)(v;q23.3), t(6;9), inv(3) or t(3;3); AML mit BCR-ABL1).

‡ nur als ungünstig einzustufen, wenn keine als günstig eingestuften Aberrationen vorliegen, d.h. in Anwesenheit günstiger Veränderungen geben diese den Ausschlag für eine Einstufung in die günstige Risikogruppe

Döhner H, Estey E, Grimwade D et al.: Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 2016.